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cc:

Subject: Group 5 ZDDP HPV Submission

HPV Test Plan Submission from the American Chemistry Council Petroleum Additives HERTG - HPV Registration Number

Three documents (1. cover letter, 2. test plan and 3. robust summaries) are attached to this e-mail for the HERTG Zinc Dialkyldithiophosphate category. If you have any questions or comments, please feel free to contact me. Below, my contact information is listed. Thank you very much. Sarah McLallen

(See attached file: Group 5 (All Docs).zip)

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- Group 5 (All Docs).zip

November 11, 2002

By Mail

Christine Todd Whitman, Administrator
US EPA
PO Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program – Test Plan Submission from HERTG
Registration Number

Dear Administrator Whitman:

The American Chemistry Council Petroleum Additives Panel (Panel) Health, Environmental, and Regulatory Task Group (HERTG) submits for review and public comment its test plan report, as well as related robust summaries, for the "*Zinc Dialkyldithiophosphate*" category of chemicals under the Environmental Protection Agency's High Production Volume (HPV) Chemical Challenge Program. The HERTG understands that there will be a 120-day review period for the test plan report and that all comments generated by or provided to EPA will be forwarded to the HERTG for consideration.

The zinc dialkyldithiophosphates in this category, which are used as petroleum lubricant additives, are characterized by having structural similarities and limited reactivity, low biological activity, and limited water solubility. Based upon the data reviewed in the attached report, the HERTG concludes that the physicochemical and toxicological properties of the proposed zinc dialkyldithiophosphate category members are similar and follow a regular pattern as a result of structural similarity. Thus, HERTG believes these twelve chemicals meet the EPA definition of a chemical category and will test them in accordance with the test plan summarized in the attached report. The twelve chemicals in the zinc dialkyldithiophosphate category are as follows:

- Phosphorodithioic acid, mixed O,O-bis(1,3-dimethylbutyl and iso-propyl) esters, zinc salts – (CAS # 84605-29-8), referred to as "mixed isopropyl and 1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and pentyl) esters, zinc salts – (CAS # 68457-79-4), referred to as "mixed isobutyl and pentyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3-dimethylbutyl) esters, zinc salts – (CAS # 68784-31-6), referred to as "mixed sec-butyl and 1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and isooctyl) esters, zinc salts – (CAS # 113706-15-3), referred to as "mixed sec-butyl and isooctyl derivative"
- Phosphorodithioic acid, O-(2-ethylhexyl) O-isobutyl ester, zinc salt – (CAS # 26566-95-0), referred to as "mixed isobutyl and 2-ethylhexyl derivative"

- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and isooctyl and pentyl) esters, zinc salts – (CAS # 68988-46-5), referred to as “mixed isobutyl, pentyl and isooctyl derivative”
- Phosphorodithioic acid, O,O-bis(1,3-dimethylbutyl) ester, zinc salt – (CAS # 2215-35-2), referred to as “1,3-dimethylbutyl derivative”
- Phosphorodithioic acid, O,O-bis(2-ethylhexyl) ester, zinc salt – (CAS# 4259-15-8), referred to as “2-ethylhexyl derivative”
- Phosphorodithioic acid, O,O-bis(isooctyl) ester, zinc salt – (CAS# 28629-66-5), referred to as “isooctyl derivative”
- Phosphorodithioic acid, O,O-diisodecyl ester, zinc salt – (CAS # 25103-54-2), referred to as “diisodecyl derivative”
- Phenol, dodecyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 54261-67-5), referred to as “dodecylphenol derivative”
- Phenol, tetrapropenyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 11059-65-7), referred to as, “tetrapropenylphenol derivative”.

Briefly, the test plan for the HERTG zinc dialkyldithiophosphate category includes the following tests and computer modeling:

- Water solubility – The solubility of the mixed isopropyl and 1,3-dimethyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS # 4295-15-8) and the tetrapropenylphenol derivative (CAS# 11059-65-7) will be measured. Results will be bridged to other members of the category.
- Photodegradation– UV absorption data will be collected on the mixed isopropyl and 1,3-dimethyl derivative (CAS# 84605-29-8) and the isooctyl derivative (CAS# 28629-66-5) to determine whether there is a potential for direct photodegradation.
- Eugacity modeling – Environmental partitioning data for members of this category will be calculated using a Mackay Level I equilibrium partitioning model and provided in robust summaries.
- Acute fish toxicity – Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8), and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.
- Acute invertebrate toxicity – Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8), and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.
- Alga toxicity - Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8), and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.

As HERTG developed this test plan, HERTG considered carefully and tried to limit how many animals might be required for tests included in the proposed plan and conditions to

which the animals might be exposed. As noted above, a minimal amount of animal testing is proposed. The HERTG believes that the concerns of some non-governmental organizations about animal welfare have been fully considered and that use of animals in this proposed test plan has been minimized.

Thank you in advance for your attention to this matter. If you have any questions regarding the test plan report or the robust summaries, or HERTG's activities associated with the Challenge Program, please contact Sarah McLallen at 703-741-5607 (telephone), 703-741-6091 (telefax) or Sarah_McLallen@americanchemistry.com (e-mail).

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

cc: HERTG members

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HIGH PRODUCTION VOLUME (HPV)

CHALLENGE PROGRAM

TEST PLAN

For

ZINC DIALKYLDITHIOPHOSPHATE CATEGORY

Prepared by
The American Chemistry Council
Petroleum Additives Panel
Health, Environmental, and Regulatory Task Group

September 24, 2002

LIST OF MEMBER COMPANIES IN THE
HEALTH, ENVIRONMENTAL AND REGULATORY TASK GROUP

The Health, Environmental, and Regulatory Task Group (HERTG) of the American Chemistry Council Petroleum Additives Panel includes the following member companies:

BP plc

Chevron Oronite Company, LLC

Crompton Corporation

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

Rhodia, Inc. (formerly Albright & Wilson Americas Inc.)

EXECUTIVE SUMMARY

The American Chemistry Council Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its member companies, hereby submit for review and public comment their test plan for the “*Zinc dialkyldithiophosphate*” category of chemicals under the United States Environmental Protection Agency High Production Volume (HPV) Chemical Challenge Program. This report should be read in its entirety in order to obtain a complete understanding of the chemical category and proposed testing.

Zinc dialkyldithiophosphate Category. Relying on several factors specified in the EPA guidance document on “Development of Chemical Categories in the HPV Challenge Program,” in which use of chemical categories is encouraged, the following twelve closely related chemicals constitute a chemical category:

- Phosphorodithioic acid, mixed O,O-bis(1,3-dimethylbutyl and iso-propyl) esters, zinc salts – (CAS # 84605-29-8), referred to as “mixed isopropyl and 1,3-dimethylbutyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and pentyl) esters, zinc salts – (CAS # 68457-79-4), referred to as “mixed isobutyl and pentyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3-dimethylbutyl) esters, zinc salts – (CAS # 68784-31-6), referred to as “mixed sec-butyl and 1,3-dimethylbutyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and isooctyl) esters, zinc salts – (CAS # 113706-15-3), referred to as “mixed sec-butyl and isooctyl derivative”
- Phosphorodithioic acid, O-(2-ethylhexyl) O-isobutyl ester, zinc salt – (CAS # 26566-95-0), referred to as “mixed isobutyl and 2-ethylhexyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and isooctyl and pentyl) esters, zinc salts – (CAS # 68988-46-5), referred to as “mixed isobutyl, pentyl and isooctyl derivative”
- Phosphorodithioic acid, O,O-bis(1,3-dimethylbutyl) ester, zinc salt – (CAS # 2215-35-2), referred to as “1,3-dimethylbutyl derivative”
- Phosphorodithioic acid, O,O-bis(2-ethylhexyl) ester, zinc salt – (CAS# 4259-15-8), referred to as “2-ethylhexyl derivative”
- Phosphorodithioic acid, O,O-bis(isooctyl) ester, zinc salt – (CAS# 28629-66-5), referred to as “isooctyl derivative”
- Phosphorodithioic acid, O,O-diisodecyl ester, zinc salt – (CAS # 25103-54-2), referred to as “diisodecyl derivative”
- Phenol, dodecyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 54261-67-5), referred to as “dodecylphenol derivative”
- Phenol, tetrapropenyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 11059-65-7), referred to as, “tetrapropenylphenol derivative”.

Although the TSCA Inventory Update Rule (IUR) reports list 1990 production volumes for “Phenol, dodecyl (*straight-chain* C12)-, hydrogen phosphorodithioate, zinc salt” (CAS # 54261-67-5), it is typical for additive manufacturers to synthesize the *branched* C12 tetrapropenyl (CAS # 11059-65-7) congener using propylene tetramer. Propylene tetramer is a distilled product manufactured from oligomerization of 1-propene under acid catalysis conditions. Commercial propylene tetramer is a range of C10-C15 olefins with the C12 propylene tetramer isomer being ~60 wt-% of the total. Although study reports may identify the dodecyl derivative as the test article, the tetrapropenyl derivative is and always has been the prepared chemical. Therefore, in this test plan the presented data for CAS registry numbers 54261-67-5 and 11059-65-7 should be considered interchangeable and referred to as the same chemical species.

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity. Zinc dialkyldithiophosphates are used as multi-functional anti-wear and anti-oxidation inhibitor performance components in passenger motor oils, diesel engine oils and industrial oils such as hydraulic lubricants. All substances in this category consist of alkyl (C3-C12) or alkaryl (C12 alkylphenol) substituted phosphorodithioic acid structures complexed with zinc. Zinc dialkyldithiophosphates are manufactured and distributed in commerce in highly refined lubricant base oil (IP 346 DMSO extractables < 3%). The oil is added during the neutralization of the dithiophosphate alkyl esters intermediate with zinc oxide. The oil acts as a solvent in the reaction, manages the viscosity and improves consistency of the final product. The zinc dialkyldithiophosphates are never isolated from base oil at any time during their life cycle. Hence, all testing for environmental fate, aquatic toxicity and health effects was performed on zinc dialkyldithiophosphates in highly refined lubricant base oil.

Similarity of Physicochemical Properties. The similarity of the physicochemical properties of these substances parallels their structural similarity. Zinc dialkyldithiophosphates are amber colored viscous liquids containing 10-15 wt-% highly refined lubricating base oil (representative lubricating base oil CAS registry numbers are 64742-54-7 and 64741-88-4). The physicochemical properties of zinc dialkyldithiophosphates largely reflect those of base oil. These materials are relatively high molecular weight, low vapor pressure, high viscosity, and poorly water soluble

Fate and Transport Characteristics. The zinc dialkyldithiophosphates are formulated for use in oils and have low water solubility. Solubility testing will be conducted on representative low and high molecular weight members of this category to confirm available data. Members of this category have been shown to be poorly biodegradable. Adequate biodegradation data exist for two commercial oil-based samples of the zinc dialkyldithiophosphate category. Bridging will be used to fill the remaining data gaps for the other ten substances. Available literature and historical information indicates that these materials are stable and are not susceptible to hydrolysis under normal conditions. These materials are known to be thermally labile at temperatures >120°C. This decomposition mechanism is key to how they provide anti-wear and anti-oxidation performance enhancements in engine oils. A search of the chemical literature has shown no known photochemical pathways, therefore photodegradation is not expected to cause significant physical degradation of zinc dialkyldithiophosphates. Nevertheless, the UV absorption data will be collected on 2 representative members of the category. If feasible, first

order reaction rates will also be calculated for chemicals identified to have a potential for direct photolysis in water.

Toxicological Similarity. The zinc dialkyldithiophosphates have a long history of use in lubricants and published and unpublished aquatic toxicity data is available for many of the members in this category. There is a wide variability in results among closely related members and even for the same chemical in this category. There are many contributing factors for this apparent variability in data, which are discussed later in this document. Review of existing published and unpublished mammalian toxicity test data for commercial samples of zinc dialkyldithiophosphates in highly refined lubricant base oil suggest that the toxicity profiles of these materials are similar. Data obtained from the proposed additional testing will further characterize of the toxicity endpoints in the HPV Challenge Program for all members within this category.

Aquatic Toxicology. Acute fish, invertebrate, and alga toxicity data for zinc dialkyldithiophosphates in highly refined lubricant base oil were reviewed, and the findings show a large variability in data. Additional testing is proposed to characterize the aquatic toxicity potential for members of this category.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity of zinc dialkyldithiophosphates in highly refined lubricant base oil were reviewed, and the findings indicate a low concern for acute toxicity. No additional acute mammalian toxicity testing is proposed.

Mammalian Toxicology - Mutagenicity. Data from bacterial reverse mutation assays, *in vitro* mutation assays in mammalian cells and *in vivo* chromosome aberration studies were reviewed. Findings indicate that commercial samples of zinc dialkyldithiophosphates in highly refined lubricant base oil have a low potential for inducing genetic toxicity. Due to the similarity of structure and physicochemical properties, the existing data can be bridged to the other members of the category where information is lacking. As a result, the category is adequately tested for mutagenicity, and no additional collection of genetic toxicity data is proposed.

Mammalian Toxicology - Systemic Toxicity. Data from several repeated-dose toxicity studies using commercial samples of zinc dialkyldithiophosphates in highly refined lubricant base oil were reviewed. Repeated dermal exposure to experimental animals resulted in moderate-to-severe dermal irritation, behavioral distress, body weight loss and emaciation, reduction in hematological parameters and adverse effects on male reproductive organs. Oral administration caused significant gastric irritation and related gastrointestinal disturbances, signs of distress but with no evidence of adverse effects on male reproductive organs. Bridging will be used to satisfy repeated dose data gaps for those zinc dialkyldithiophosphates in highly refined lubricant base oil where the carbon chain lengths/molecular weights are similar, yet lack subchronic toxicity information. No additional repeated-dose systemic toxicity testing is proposed.

Mammalian Toxicology - Reproductive and Developmental Toxicity. Data from a study on the 2-ethylhexyl derivative in highly refined lubricant base oil indicates a low concern for reproduction/ developmental toxicity. Furthermore, an epidemiological study on workers exposed to oil-based zinc dialkyldithiophosphates (range C4-C8) in an additive manufacturing plant revealed no adverse effects on worker reproductive health. Review of the available information underscores the similarity of clinical and pathological findings in repeated-dose dermal toxicity studies with C4-C10 zinc dialkyldithiophosphates, as well as the absence of reproduction and developmental toxicity and the lack of untoward findings in a human epidemiological investigation. In light of the irritant properties that zinc dialkyldithiophosphates in highly refined lubricant base oil have on skin and gastrointestinal mucosa, any additional repeated dose testing would cause unnecessary distress and suffering in experimental animals, and would add no additional insight into the hazard assessment of this category of substances. Therefore, the HERTG concludes that the existing information is adequate to characterize the reproduction/developmental toxicity profile of the entire zinc dialkyldithiophosphate category. No additional repeated dose testing is proposed.

Conclusion. Based upon the data reviewed for this test plan, the physicochemical, environmental fate, and toxicological properties of the proposed zinc dialkyldithiophosphate category members in highly refined lubricant base oils are similar and/or follow a regular, predictable pattern. Variability was seen in the aquatic toxicity data between the same or closely related members of the category. This is attributed to the test methodology used and is not due to a difference in the nature or chemistry of the category members. Further testing is therefore proposed to confirm this hypothesis. Therefore, the EPA definition of a chemical category has been met, and the twelve chemicals that constitute the zinc dialkyldithiophosphate category will be tested in accordance with the test plan summarized below.

Test Plan. The test plan for the zinc dialkyldithiophosphate category includes the following tests or computer modeling:

- Water solubility – The solubility of the mixed isopropyl and 1,3-dimethyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS # 4295-15-8) and the tetrapropenylphenol derivative (CAS# 11059-65-7) will be measured. Results will be bridged to other members of the category.
- Photodegradation– UV absorption data will be collected on the mixed isopropyl and 1,3-dimethyl derivative (CAS# 84605-29-8) and the isooctyl derivative (CAS# 28629-66-5) to determine whether there is a potential for direct photodegradation.
- Fugacity modeling – Environmental partitioning data for members of this category will be calculated using a Mackay Level I equilibrium partitioning model and provided in robust summaries.
- Acute fish toxicity – Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8), and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.
- Acute invertebrate toxicity – Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8),

and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.

- Alga toxicity - Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8), and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.

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1.0 INTRODUCTION

In March 1999, the American Chemistry Council (formerly the Chemical Manufacturers Association) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address data needs for certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address physico-chemical, environmental, aquatic and health effects testing information for the following twelve substances:

- Phosphorodithioic acid, mixed O,O-bis(1,3-dimethylbutyl and iso-propyl) esters, zinc salts – (CAS # 84605-29-8), referred to as “mixed isopropyl and 1,3-dimethylbutyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and pentyl) esters, zinc salts – (CAS # 68457-79-4), referred to as “mixed isobutyl and pentyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3-dimethylbutyl) esters, zinc salts – (CAS # 68784-31-6), referred to as “mixed sec-butyl and 1,3-dimethylbutyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and isooctyl) esters, zinc salts – (CAS # 113706-15-3), referred to as “mixed sec-butyl and isooctyl derivative”
- Phosphorodithioic acid, O-(2-ethylhexyl) O-isobutyl ester, zinc salt – (CAS # 26566-95-0), referred to as “mixed isobutyl and 2-ethylhexyl derivative”
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and isooctyl and pentyl) esters, zinc salts – (CAS # 68988-46-5), referred to as “mixed isobutyl, pentyl and isooctyl derivative”
- Phosphorodithioic acid, O,O-bis(1,3-dimethylbutyl) ester, zinc salt – (CAS # 2215-35-2), referred to as “1,3-dimethylbutyl derivative”
- Phosphorodithioic acid, O,O-bis(2-ethylhexyl) ester, zinc salt – (CAS# 4259-15-8), referred to as “2-ethylhexyl derivative”
- Phosphorodithioic acid, O,O-bis(isooctyl) ester, zinc salt – (CAS# 28629-66-5), referred to as “isooctyl derivative”
- Phosphorodithioic acid, O,O-diisodecyl ester, zinc salt – (CAS # 25103-54-2), referred to as “diisodecyl derivative”
- Phenol, dodecyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 54261-67-5), referred to as “dodecylphenol derivative”
- Phenol, tetrapropenyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 11059-65-7), referred to as, “tetrapropenylphenol derivative”.

Although the TSCA Inventory Update Rule (IUR) reports list 1990 production volumes for “Phenol, dodecyl (*straight-chain* C12)-, hydrogen phosphorodithioate, zinc salt” (CAS # 54261-67-5), it is typical for additive manufacturers to synthesize the *branched* C12 tetrapropenyl

congener (CAS # 11059-65-7) using propylene tetramer. Therefore, the tetrapropenyl derivative is included in this category analysis document and testing plan so that the physicochemical, environmental fate, aquatic toxicity and health effects of all high production volume zinc dialkyldithiophosphates in highly refined lubricant base oil will be captured. Consequently, data submitted in this category justification and testing plan for CAS # 54261-67-5 and 11059-65-7 should be considered interchangeable and referred to as the same chemical species.

An analysis of the available data on these chemicals supports the designation of the zinc dialkyldithiophosphates as a “chemical category” as provided in the EPA guidance document entitled, “Development of Chemical Categories in the HPV Challenge Program”. This document provides the basis for that determination, indicates the findings of the data review process, and sets forth a proposed testing plan to satisfy parts of the required test battery for endpoints without data that would be considered adequate under the program.

EPA guidance on the HPV Chemical Challenge Program indicates that the primary purpose of the program is to encourage “the chemical industry . . . to voluntarily compile a Screening Information Data Set (SIDS) on all chemicals on the US HPV list.” (EPA, “Development of Chemical Categories in the HPV Challenge Program,” p. 1) At the same time, EPA recognizes that the “large number of chemicals to be tested [about 2800 HPV chemicals] makes it important to reduce the number of tests to be conducted, *where this is scientifically justifiable*.” (*Id.*, p. 1) [emphasis added] The next part of the guidance explains where this would be scientifically justifiable:

One approach is to test closely related chemicals as a group, or category, rather than test them as individual chemicals. In the category approach, *not every chemical needs to be tested for every SIDS endpoint*. However, *the test data finally compiled* for the category must prove adequate to support a screening level hazard-assessment of the category and its members. That is, the *final data set* must allow one to estimate the hazard for the untested endpoints, *ideally* by interpolation between and among the category members. In certain cases, where toxicity is low and no upward trend is expected, extrapolation to the higher category members may be acceptable. (*Id.*, p. 1) [emphasis added].

EPA guidance goes on to state, “The use of categories is encouraged in the Challenge Program and will have a number of benefits.” (*Id.*, p. 1) Among the benefits identified in the guidance for the use of categories are “a reduction in testing will result in fewer animals used to test a category of chemicals as opposed to doing each test on each individual chemical,” and “there will be . . . economic savings since less testing may be needed for chemicals considered as a category.” (*Id.*, p. 1) That guidance also states that categories “accomplish the goal of the Challenge Program – to obtain screening level hazard information – through the strategic application of testing to the category.” (*Id.*, p. 2)

A similarly stated intent “to reduce the number of tests to be conducted, *where this is scientifically justifiable*” was articulated by the Agency in its draft guidance document titled, “The Use of Structure Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program.” [emphasis added].

The EPA “Chemical Categories” guidance sets forth a definition of what constitutes a “chemical category, for the purposes of the Challenge Program”. Specifically, that definition states that a chemical category under the HPV Challenge Program “is a group of chemicals whose physicochemical and toxicological properties *are likely to* be similar *or* follow a regular pattern as a result of structural similarity.” (*Op. Cit.*, p. 2) [emphasis added].

According to the guidance, what is important is that the “structural similarities [among members of the group] *may* create a predictable pattern *in any* or all of the following parameters: physicochemical properties, environmental fate and effects, and human health effects.” (*Id.*, p. 2) [emphasis added]. Thus, it is not necessary for the chemicals in a category to be similar in all respects. Nor must there be conclusive proof that the chemicals in the postulated category will behave identically across all relevant parameters. All that is required for an acceptable category under the HPV Challenge Program is that there be a *likelihood* of similarity of physicochemical and toxicological properties or a *likelihood* that the chemicals will in some pertinent respect follow a regular pattern as a result of their structural similarity.

In identifying the zinc dialkyldithiophosphate category, the six-step process set out in the EPA guidance on category development was followed. As the information below indicates, the zinc dialkyldithiophosphate chemicals clearly satisfy the standards established in that guidance for use of a chemical category:

Step 1: group structurally similar chemicals into a putative category

Step 2: gather relevant published and unpublished literature for each member of the category

Step 3: evaluate the compiled data for adequacy in accordance with the EPA guidance documentation

Step 4: construct matrices of SIDS endpoints versus category members arranged so as to indicate the structural progression of the category (in this case, by increasing molecular weight)

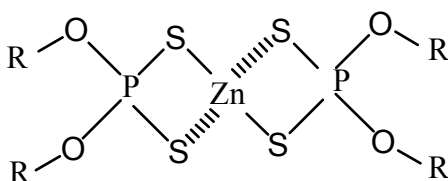
Step 5: evaluate the data to determine whether there is a correlation between category members for each SIDS endpoint

Step 6: make available to EPA, and to the public for review, this test plan including the foregoing category definition and rationale and the following data assessment with the proposed testing scheme for the zinc dialkyldithiophosphates.

2.0 CHEMISTRY OF ZINC DIALKYLDITHIOPHOSPHATES

2.1 Description

Zinc dialkyldithiophosphates consist of a phosphorodithioic acid structure with alkyl or alkaryl ester substituent groups. The alkyl groups are saturated hydrocarbon chains that vary in length (C3-C10) and in the extent of branching. An idealized structure for the zinc dialkyldithiophosphate component is shown below.



R = C3 – C10 (linear and/or branched) alkyl or C12 (branched) alkaryl

The chemical names and CAS numbers for the members of the zinc dialkyldithiophosphate category are presented in Table 1 and the chemical structures in Table 2. These substances are prepared by reacting phosphorous pentasulfide (P_2S_5) with one or more primary or secondary C3-C10 branched or linear alcohols to form the phosphorodithioic acid ester. The only exception is the alkaryl dithiophosphate where the alcohol moiety is tetrapropenylphenol. The dithiophosphoric acid ester is further diluted with 10-15 wt-% highly refined lubricating base oil (typical CAS #s 64742-54-7 and 64741-88-4) before it is neutralized with zinc oxide. The oil acts as a solvent in the neutralization reaction, manages the viscosity of the final product and improves consistency. The zinc complex that is formed upon neutralization is not a salt in the traditional sense, since the Zn-S bond is more coordinate covalent in character than ionic.

It should be noted that additive manufacturers synthesize the *branched* alkaryl C12 tetrapropenyl congener using propylene tetramer. Propylene tetramer is a distilled product manufactured from oligomerization of 1-propene under acid catalysis conditions. Commercial propylene tetramer is a range of C10-C15 olefins with the C12 propylene tetramer isomer being ~60 wt-% of the total. Although study reports may identify the dodecyl derivative as the test article, the tetrapropenyl derivative is and always has been the prepared chemical.

2.2 Physiochemical Properties

The physicochemical properties of the members of the zinc dialkyldithiophosphate category are presented in Table 3. Zinc dialkyldithiophosphate produced for use as lubricating petroleum additive are manufactured and distributed in 10-15 wt-% highly refined lubricating base oils. The

highly refined lubricating base oil used in the manufacture of the zinc dialkyldithiophosphates cannot be removed without altering the structural and physicochemical character of the zinc dialkyldithiophosphate molecules. Therefore, many of the physicochemical properties presented are qualitative estimates.

2.2.1 Alkyl Chain Length and Molecular Weight

As discussed above, the members of the zinc dialkyldithiophosphate category contain alkyl chain lengths that range from C3-C10, or tetrapropenylphenol (range = C10-C15, C12 enriched). It is common for zinc dialkyldithiophosphates to contain mixed alkyl esters (e.g., C4, C5), although derivatives with single chain lengths (e.g., C8) are included in the category. Alkyl groups can be linear or branched. Two members of the category contain alkylphenol ester side-chains. As a result of this diversity in alkyl side chain length, the molecular weight distribution for the members of the category is broad, 578 to 1303 gm/mol. Due to the predominant influence of carbon chain length on molecular weight, the members of the category are arrayed in all tables in order of increasing carbon chain length.

2.2.2 Melting Point and Boiling Point

Zinc dialkyldithiophosphates as manufactured and distributed in commerce in highly refined lubricating base oil, are high viscosity liquids at ambient temperature. However, at elevated temperatures ($> 120^{\circ}\text{C}$), zinc dialkyldithiophosphates become unstable and degrade.

2.2.3 Vapor Pressure

Due to the technical difficulty in isolating intact zinc dialkyldithiophosphates from the highly refined lubricating base oil, vapor pressure has not been measured on the pure chemical. However, vapor pressure measurements have been performed by a consortium member company on C8 ester zinc dialkyldithiophosphate (90% in base oil) and on the pure highly refined lubricating base oil. A vapor pressure of < 0.5 mmHg at 25°C and 60°C was measured for both the materials. This suggests that the vapor pressure for the zinc dialkyldithiophosphate is less than 0.5 mmHg.

2.2.4 Water Solubility and Octanol-Water Partition Coefficients

The zinc dialkyldithiophosphates are formulated for use in oils and have very low water solubility. Unpublished company data for a commercial zinc dialkyldithiophosphate with an alkyl group less than C8 indicates a water solubility of 1.6 mg/L ¹. Historically, the zinc dialkyldithiophosphates are

¹ Information Review: Zinc Dialkyl Dithiophosphates. CRCS, Inc. Prepared under EPA Contract No. 68-01-6650 for TSCA Interagency Testing Committee. October 31, 1984.

generally regarded to be poorly soluble in water. In order to adequately define the solubility range of the members of this category, water solubility testing will be conducted on selected members of this category:

- the mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8) in highly refined lubricating base oil, which contains the lowest molecular weight substance and the shortest alkyl side chain (C3) in the category. This is expected to be the most soluble member and should provide the upper-bound solubility value for this category
- the 2-ethylhexyl derivative (CAS # 4259-15-8) in highly refined lubricating base oil which represents a higher molecular weight member of this category
- the tetrapropenylphenol derivative (CAS # 11059-65-7) in highly refined lubricating base oil which represents a higher molecular weight alkaryl member of this category.

Unpublished company data on a commercial zinc dialkyldithiophosphate with a carbon chain length of less than eight yielded a log P value of 2.49¹. Longer chain materials are likely to have higher octanol/water partition coefficients. The log P is a measure of the lipophilicity of a substance and is used as a surrogate indicator of the potential of a chemical substance to bioaccumulate in aquatic organisms. While Log P is a good predictor of bioaccumulation for nonpolar organic compounds, the mechanisms for uptake and depuration of metals and metal compounds are very complex and variable. For metal compounds, the Log P data are not indicative of the bioaccumulation potential. In view of the above, no further testing for log P is proposed.

3.0 USES OF ZINC DIALKYLDITHIOPHOSPHATES

Zinc dialkyldithiophosphates are used to formulate finished lubricating oils including all types of automotive and diesel engine crankcase, industrial oils and hydraulic fluids. They are used as anti-wear inhibitors to reduce wear in engines and hydraulic equipment parts, and also act as antioxidants. Zinc dialkyldithiophosphates are generally sold to finished oil blenders in additive packages, where the concentration ranges from 1 to 20 wt-%. These additive packages are then blended into finished oils where the typical concentration of zinc dialkyldithiophosphate ranges from 0.1 to 10 wt-% in the finished oil.

Zinc dialkyldithiophosphates are manufactured and blended into additive packages at plants owned by members of the HERTG. Finished lubricants are blended at facilities owned by our customers. Additive packages are shipped to customers in ships, iso-containers, railroad tank

cars, tank trucks or in 55-gallon steel drums. The additive packages are stored in bulk storage tanks at the customer blending sites. Finished oils are blended by pumping the lubricating oil blend stocks and the additive package from their storage tanks through computer controlled valves that meter the precise delivery of the components into a blending tank. After blending, the finished lubricant products are sold in bulk and shipped in tank trucks to large industrial users, such as manufacturing facilities and facilities that service truck fleets and passenger motor vehicles. Finished lubricants are also packaged into 55-gallon drums, 5-gallon pails, and one-gallon and one-quart containers for sale to smaller industrial users. Sales of lubricants in one-gallon and one-quart containers to consumers at service stations or retail specialty stores also occur.

Based on these uses, the potentially exposed populations include (1) workers involved in the manufacture of zinc dialkyldithiophosphates, blending them into additive packages, and blending the additive packages into finished lubricants; (2) quality assurance workers who sample and analyze these products to ensure that they meet specifications; (3) workers involved in the transfer and transport of zinc dialkyldithiophosphates, additive packages or finished lubricants that contain them; (4) mechanics who may come into contact with both fresh and used lubricants while working on engines or equipment; (5) gasoline station attendants and consumers who may periodically add lubricating oil to automotive crankcases; and (6) consumers who may change their own automotive engine oil. The most likely route of exposure for these substances is skin and eye contact. Manufacturing, quality assurance, and transportation workers will likely have access to engineering controls and wear protective clothing to minimize exposure. Mechanics wear protective clothing, but often work without gloves or eye protection. Gasoline station attendants and consumers often work without gloves or other protective equipment. The most likely source of environmental exposure is accidental spills at manufacturing sites and during transport.

4.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA

4.1 Environmental Fate Data

4.1.1 Physicochemical Properties Relevant to Environmental Fate

In order to understand the environmental fate of a substance, one must understand how that substance can potentially partition among environmental compartments (i.e., air, soil, sediment, suspended sediment, water, and biota). The physicochemical properties of a substance influence the way in which a substance will degrade. The important environmental degradation pathways include biodegradation, hydrolysis, and photodegradation. Biodegradation is a measure of the potential of a compound to be degraded by microorganisms. Hydrolysis is a reaction in which a water molecule or hydroxide ion substitutes for another atom or group of atoms present in an organic

molecule. Photodegradation is the degradation of a chemical compound as a result of absorption of solar radiation.

The physicochemical properties of the parent substance will influence the way in which these substances may partition among environmental compartments. Substances characterized by a low vapor pressure do not partition into air to any great extent. Similarly, substances that are characterized by low water solubility do not partition extensively into water. Substances that do not partition into air and water to any great extent tend to partition into soil and sediments.

4.1.2 Biodegradability

4.1.2.1 Test Methodologies

Chemical biodegradation involves a series of microbially-mediated reactions that may require many kinds of microorganisms acting together to degrade the parent substance. There are several standard test methods, which measure primary degradation (i.e., loss of parent chemical) or ultimate degradation (i.e., complete utilization of the substance to produce carbon dioxide, water, mineral salts, and microbial biomass). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals, infrared absorbance, or by a chemical-specific detection method. Ultimate degradation (also called mineralization) can be determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on an elemental analysis of the chemical under investigation.

4.1.2.2 Summary of Available Data

Biodegradation data for commercial samples of two of the zinc dialkyldithiophosphates in highly refined lubricating base oil are summarized in Table 4.

The biodegradability of the dodecylphenol derivative (CAS # 54261-67-5) in highly refined lubricating base oil was evaluated using the Modified Sturm Test (OECD Guideline 301B, *CO₂ Evolution Test*) and the *Manometric Respirometry Test* (OECD Guideline 301F). After 28 days in each test, the extent of biodegradation was 5.9% based on carbon dioxide evolution and 4.2% based on theoretical oxygen demand, respectively.

The Modified Sturm Test (OECD Guideline 301B, *CO₂ Evolution Test*) was used to evaluate the biodegradability of the mixed isopropyl and 1,3-dimethylbutyl derivative (CAS # 84605-29-8) in highly refined lubricating base oil. After the 28-day test, the extent of biodegradation was 5.9% based on carbon dioxide evolution.

4.1.2.3 Data Assessment and Test Plan for Biodegradability

Adequate biodegradation data exist for two of twelve substances in the zinc dialkyldithiophosphate category including the lowest molecular weight (CAS# 84605-29-8) and the highest molecular weight (CAS# 54261-67-5) members. The results indicate that these substances are poorly biodegraded irrespective of molecular weight. Therefore, these data will be used to bridge to all intermediate molecular weight category members, thereby characterizing the biodegradability of the entire category.

4.1.3 Hydrolysis

4.1.3.1 Test Methodologies

The potential for a substance to hydrolyze in water is assessed as a function of pH (OECD Guideline 111, *Hydrolysis as a Function of pH*²). When an organic molecule undergoes hydrolysis, a nucleophile (water or hydroxide ion) attacks an electrophile and displaces a leaving group (e.g., halogen, phenoxide).³ Potentially hydrolyzable groups include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters³. The lack of a suitable leaving group renders compounds resistant to hydrolysis and the lack of water solubility make testing for hydrolysis unfeasible.

4.1.3.2 Summary of Available Data

Zinc dialkyldithiophosphates are formulated in oil and are hydrolytically stable under normal conditions. This is documented in various studies that have been conducted to study the hydrolytic stability and hydrolysis pathways for the zinc dialkyldithiophosphates. The studies were carried out by heating the zinc dialkyldithiophosphates at 85°C to achieve hydrolysis⁴. These substances have little, if any, potential for hydrolysis under environmentally relevant conditions.

4.1.3.3 Data Assessment and Test Plan for Hydrolysis

Since available literature information and historical use of these substances in petroleum additive formulations indicates that these materials are not subject to hydrolytic degradative mechanisms under normal conditions, no hydrolysis testing is proposed.

² Organization for Economic Cooperation and Development (OECD) (1993) OECD Guidelines for Testing of Chemicals. OECD. Paris, France.

³ W. Lyman et al. (1990) *Handbook of Chemical Estimation Methods*. Chapter 8.

⁴ Burn A.J. et al. Analysis of the Hydrolytic Stability of Zinc(II) O,O-Dialkyl Dithiophosphates as a Function of the Nature of the Alkyl Groups by ³¹P NMR Spectroscopy. J. Chem. Soc. Perkin Trans. 2, 1992.

4.1.4 Photodegradation

4.1.4.1 Test Methodologies

Photodegradation can occur as a result of direct and indirect mechanisms. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. In comparison, indirect photodegradation also requires light energy as well as a series of chemical reactions that include a reaction of the parent molecule with hydroxyl radicals.

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may lead to its transformation. Simple chemical structures can be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline⁵.

To develop information or data that will characterize the potential of products in this category to undergo direct photochemical degradation, the existing product chemical composition data will be evaluated to select a subset of chemicals that adequately represents products in this category. The selection process will consider chemical carbon number range and structures.

4.1.4.2 Summary of Available Data

There are no published or unpublished photodegradation studies for members of the zinc dialkyldithiophosphate category. An initial review of the members of the zinc dialkyldithiophosphate category suggests that category members do not contain bonds that have a high potential to absorb UV light above 290 nm.

4.1.4.3 Data Assessment and Test Plan for Photodegradation

HPV Challenge Program guidance suggests that photodegradation testing be performed on each member of a category or adequate data used to bridge from selected category members with data to the remaining members that have not been tested. The UV light absorption spectra will be taken for the mixed isopropyl and 1,3-dimethylbutyl derivative (CAS # 84605-29-8) and the isooctyl derivative (CAS # 28629-66-5). The results obtained for these substances will be

⁵Zepp, R. G., and D. M. Cline. 1977. Rates of Direct Photolysis in the Aqueous Environment. *Environ. Sci. Technol.* 11:359-366.

indicative of whether direct photolysis is a relevant pathway for members of this category. If feasible, first order reaction rates will also be calculated for chemicals identified to have a potential for direct photolysis in water. The results of the calculations will be summarized in a technical discussion for this endpoint. Indirect photodegradation as a result of hydroxyl radical interaction is not a significant pathway as these substances are not volatile and will not exist in the vapor phase.

4.1.5 Fugacity Modeling

4.1.5.1 Modeling Methodologies

Fugacity-based multimedia fate modeling compares the relative distribution of chemicals among environmental compartments. A widely used model for this approach is the EQC model⁶.

There are multiple levels of the EQC model. In the document, “Determining the Adequacy of Existing Data”, EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. The EQC Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized environment. EQC Level III model uses these parameters to evaluate chemical distribution based on emission rates into air, water, and soil, as well as degradation rates in air, water, soil, and sediment.

4.1.5.2 Summary of Available Data

There are no published or unpublished fugacity-based multimedia fate modeling data for members of the zinc dialkyldithiophosphate category. All of the members of this category have low vapor pressure and low water solubility indicating that they will not tend to partition into the air or water to any great extent.

4.1.5.3 Test Plan for Fugacity

The relative distribution of substances within this category among environmental compartments will be evaluated using the Level I model. Data developed using a Level I model can then be used for simple comparative purposes across several substances. EQC Level III will not be used for this evaluation because appropriate emission levels are as yet unknown. Because of the physical nature of the substances in this category, a Level I data set will be as equally robust as a Level

⁶. Mackay, D., A. Di Guardo, S. Paterson, and C. E. Cowan. 1996. Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ.

III data set and can then be used to assess the potential partitioning behavior of zinc dialkyldithiophosphate category members in the environment.

4.2 ECOTOXICOLOGY DATA

4.2.1 Aquatic Ecotoxicity Testing

4.2.1.1 Test Methodologies

Acute aquatic ecotoxicity tests are usually conducted with three species that represent three trophic levels in the aquatic environment: fish, invertebrates, and algae. The fish acute toxicity test (OECD Guideline 203, *Fish, Acute Toxicity Test*) establishes the lethality of a substance to a fish during a 96-hour exposure period. The acute invertebrate test (OECD Guideline 202, *Daphnia sp., Acute Immobilization Test and Reproduction Test*) establishes the lethality of a substance to an invertebrate, typically a daphnid (*Daphnia magna*), during a 48-hour exposure period. The alga growth inhibition test (OECD Guideline 201, *Alga, Growth Inhibition Test*) establishes the potential of a substance to inhibit alga growth, typically using the freshwater unicellular green algae, *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*), during a 96-hour exposure period.

Three test methodologies are commonly used to conduct aquatic toxicity tests; i.e., flow-through, static, and static renewal tests.

In *flow-through tests*, organisms are continually exposed to fresh chemical concentrations in each treatment level in the incoming water and there is greater assurance than with other test methods that the exposure levels and water quality remains constant throughout the test. Although flow-through testing is the preferred method, it is only applicable for chemicals that have adequate water solubility for testing.

In *static tests*, organisms are exposed in the test medium that is not replaced for the duration of the study. There is less assurance that the test concentrations will remain constant because test material can be adsorbed onto test chambers, degraded, volatilized, or otherwise changed during the test. Nevertheless, due to limitations of other test systems for non-volatile materials, the static test has been widely used, especially for testing organisms such as algae and *Daphnia*.

The *static-renewal test* is similar to a static test because it is conducted in still water, but the test solutions and control water are renewed periodically, usually every 24 hours. Daily test solution renewal provides a greater likelihood that the exposure concentrations will remain stable throughout the test. This is the preferred method for conducting aquatic toxicity tests for compounds such as the

zinc dialkyldithiophosphate on fish. Daily renewals cannot be done in the algae test, and usually not in *Daphnia* tests, because the process of separation and replenishment would cause a discontinuity in the alga growth rate and it can stress, coat, or entrap *Daphnia* in any surface film during renewals. OECD considers the use of static test for *Daphnia* and algae, and the use of static renewal test for fish to be appropriate for testing poorly soluble chemicals like the zinc dialkyldithiophosphate provided that test solution preparation uses water accommodated fraction or water soluble fraction methods.⁷

4.2.1.2 Test Solution Preparation

Zinc dialkyldithiophosphates are poorly water-soluble substances, and it is not possible to prepare exposure solutions for aquatic toxicity testing by direct addition of measured quantities of test material to water. Two methods⁸ are used to prepare solutions of poorly water-soluble materials for aquatic toxicity testing:

- *Water accommodated fraction* (WAF) – This is a method in which the test solution contains only that fraction of the test material (organic phase) which is retained in the aqueous phase after a period of stirring long enough to reach equilibrium, followed by a sufficient time (1-4 hours) for phase separation. The WAF (aqueous phase) will contain soluble components of the test material at levels that will be dependent on the test material loading (the amount of material added to the aqueous medium). The resulting WAF is used in the aquatic toxicity test. Ideally, a WAF consists of a water-soluble extract of test material, but it can also include a stable micro-emulsion or contain small amounts of suspended matter.
- *Water soluble fraction* (WSF) – This is a method in which a WAF is either filtered, centrifuged, or allowed to settle for a greater length of time (24 hours) than with the WAF method to remove suspended matter from the aqueous phase before being used in the aquatic toxicity test.

4.2.1.3 Reporting Toxicity Results

In both WAF and WSF tests, test material concentrations are expressed as loading rates (i.e., defined as the weight of test material added per unit volume of test medium during WAF or WSF preparation)⁹. For fish tests, endpoints can be

⁷ Organization for Economic Cooperation and Development (OECD) (2000). Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No.23, Paris, France.

⁸ American Society for Testing and Materials (1998) D6081-98, Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation.

⁹ Organization for Economic Cooperation and Development (OECD) (1999) Draft Guidance document on Aquatic Toxicity Testing of Difficult Substances. OECD, France.

expressed as median lethal loading rate (LL_{50}) when lethal effects occur to 50% of the test population or in cases where no lethal effects are observed at all loadings tested, LL_0 . In both cases, results can be expressed in mg/L and in studies where no lethality is observed, the result is expressed as LL_0 = the highest loading rate tested. For invertebrate and alga tests, endpoints are expressed as median effective loading rate (EL_{50}) or EL_0 in mg/L as discussed above.

Loading rates allow poorly water-soluble complex substances such as the zinc dialkyldithiophosphate to be compared to more readily soluble substances and /or pure chemicals on an equal basis. To allow comparison, the toxicity value is expressed as the amount of test material added per unit volume of water when preparing the WAF or WSF.

If test material exposure levels are analytically measured in the test, the endpoints can also be expressed as median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) in mg/L. EC/LC_{50} s are often not reported because it is very difficult to accurately measure test material exposure levels that can be below 1.0 mg/L.

NOTE: In this test plan, these results are reported as loading rates (EL/LL), to reflect the current reporting practices for the WAF method used in the tests. In the robust summaries, these data are presented as concentrations (EC/LC) as originally reported even though the test methods employed WAF preparation of test solutions without measurement of test material concentration.

4.2.2 Aquatic Toxicity of the Zinc Dialkyldithiophosphates

In general, the toxicity of a substance to an organism is limited by mechanisms of uptake and movement to target organs. Characteristics such as smaller molecular size and a lesser degree of ionization increase the ability of a substance to passively cross biological membranes. However, the soluble fraction of a compound in water represents the chemical fraction responsible for toxicity to aquatic organisms. Therefore, aquatic toxicity can be limited by the water solubility of a substance.

Available data and historical use information indicates that the zinc dialkyldithiophosphates formulated in highly refined lubricating base oil have limited water solubility. . However, the length of the alkyl side chains on these substances may influence their relative water solubility, and, hence, their relative toxicity. As discussed earlier in this document, the proposed water solubility testing on selected members of this category will adequately define the solubility range of the members of this category and provide guidance for conducting aquatic toxicity tests.

4.2.2.1 Summary of Available Data

The zinc dialkyldithiophosphates have a long history of use in lubricants and published and unpublished aquatic toxicity data are available for many of the members in this category. However, there is significant variability in results among closely related members and even for the same chemical in this category. A thorough review of the available data was conducted by the HERTG and it appears that the variability in data may be a result of differences in both the testing methodology and test material used. There is little or no information available on test material purity and the amount of the active material in the sample to correlate the dose-response seen in a study to the test material.

Test methodology used in older studies appears to be a significant factor in the variability seen in the data. As discussed earlier, the zinc dialkyldithiophosphates are sparingly soluble in water and direct addition of these chemicals to the exposure solution is not feasible. In some of the studies, the test material was added directly above its water solubility resulting in the presence of undissolved test material including oil droplets and surface sheen in the exposure solution. It is apparent in some of the studies that physical fouling (coating of the fish gills and trapping of smaller invertebrates) contributed to the toxicity and erratic dose response was seen. For example, higher mortality/effects were seen in the lower test concentration compared to the higher test concentration. In other studies, even though a WAF approach was used, proper techniques for separating the soluble components in the water phase were not used resulting in oil sheen in the exposure solutions. It also appears that a very vigorous stirring technique was used in some studies that resulted in an inseparable emulsion of oil in water, which significantly contributed to adverse effects in test organisms. In some of the older studies, use of a co-solvent and dissolution of the test material above its water solubility limit may have contributed to toxicity that would not be associated with the test material under normal, environmentally relevant exposure conditions. It should also be noted that since test material composition and purity information is unavailable for these studies, use of a solvent or the exposure to the test material over the limit of solubility may have resulted in preferential dissolution of the impurities resulting in adverse effects to the test organisms.

4.2.2.2 Data Assessment and Test Plan for Acute Aquatic Ecotoxicity

As discussed above, there is significant data variability in the available aquatic toxicity data. The LL50 and/or EL50 values range from <0.1 mg/L to >100 mg/L for a single or closely related chemical within the category. HERTG has therefore determined that these data are inadequate to characterize the hazard of the members of this category.

The following aquatic toxicity testing is proposed to characterize the hazards of the category members.

A commercial sample of the mixed isopropyl and 1,3-dimethylbutyl derivative (CAS # 84605-29-8) in highly refined lubricating base oil, which contains the lowest molecular weight substance and the shortest alkyl side chain (C3) in the category, will be tested for acute aquatic toxicity to fish, invertebrates, and algae. The results of these tests will be bridged to the rest of the similar substances in the category:

- Mixed isobutyl and pentyl derivative (CAS # 68457-79-4),
- Mixed sec-butyl and 1,3-dimethylbutyl derivative (CAS # 68784-31-6),
- Mixed sec-butyl and isooctyl derivative (CAS # 113706-15-3),
- Mixed isobutyl and 2-ethylhexyl derivative (CAS # 26566-95-0),
- 1,3-dimethylbutyl derivative (CAS # 2215-35-2),
- Mixed isobutyl, pentyl and isooctyl derivative (CAS # 68988-46-5),

A commercial sample of the 2-ethylhexyl derivative (CAS # 4259-15-8) in highly refined lubricating base oil will be tested for acute aquatic toxicity in fish, invertebrate and algae. The results will be bridged to other similar members of the category; the isooctyl derivative (CAS # 28629-66-5) and the diisodecyl derivative (CAS # 25103-54-2).

- A commercial sample of the tetrapropenylphenol derivative (CAS # 11059-65-7) in highly refined lubricating base oil will be tested for acute aquatic toxicity in fish, invertebrate and algae. The results will be bridged to the dodecylphenol derivative (CAS # 54261-67-5).

4.3 MAMMALIAN TOXICOLOGY DATA

4.3.1 Acute Mammalian Toxicity of the Zinc dialkyldithiophosphates

4.3.1.1 Acute Toxicity Test Methodology

Acute toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. Potential routes of exposure for acute toxicity assays include oral, dermal, and inhalation. Oral toxicity assays are conducted by administering test material to fasted animals (typically rats or mice) in a single gavage dose. Acute dermal toxicity tests are conducted by administering test material to the shaved skin on the back of the test animal (typically rats or rabbits) and allowing the test material to stay in contact with the skin application site for a specific duration (usually 24 hours). Acute inhalation toxicity assays are conducted by exposing test animals (typically rats) in a controlled atmosphere to a fixed air concentration of the test substance for a specific duration (typically 4 hours). The test material is generated as a vapor or intentionally aerosolized into respirable particles, then metered into the exposure air at the desired

concentration. Preferably, inhalation toxicity studies are conducted using either nose-only or head-only exposure to minimize potential confounding effects resulting from whole-body exposure. Whole body exposure may lead to over-prediction of inhalation toxicity hazard by increasing the body-burden of the test material through skin absorption or ingestion of test material as a consequence of grooming both during and after the inhalation exposure period.

Historically, lethality is a primary end-point of concern in acute toxicity studies, and the traditional index of oral and dermal potency is the median lethal dose that causes mortality in 50 percent of the test animals (LD_{50}). In acute inhalation studies, the traditional measurement of potency is the median lethal concentration of the test material in air that causes mortality in 50 percent of the test animals (LC_{50}). In addition to lethality, acute toxicity studies also provide insights regarding potential systemic toxicity through careful observation and recording of clinical signs and symptoms of toxicity as well as through detailed examination of tissues and organ systems.

Typically, acute oral and dermal toxicity studies are conducted using a limit dose of 5000 and 2000 mg/kg body weight, respectively, and acute inhalation toxicity studies are conducted using a limit dose of 5 mg/L for 4 hours (according to OECD and EPA testing guidelines). Prior to 1990, some acute dermal toxicity studies may have used a limit dose of 5000 mg/kg. Recently, harmonized EPA testing guidelines (August 1998) have set the limit dose for both oral and dermal acute toxicity studies at 2000 mg/kg body weight, while the recommended limit concentration for acute inhalation studies has been set at 2 mg/L for 4 hours. The limit dose test method minimizes the number of animals tested by exposing a single group of animals to a large dose (the limit dose) of the test substance. A test substance that shows little or no effects at the limit dose is considered essentially nontoxic, and no further testing is needed. If compound-related mortality is observed at the limit dose, then further testing may be necessary.

4.3.1.2 Summary of Available Data

Acute toxicity data for commercial sample of zinc dialkyldithiophosphates in highly refined lubricating base oil is summarized in Table 6. Ten members of the category have been tested by either the oral or dermal route of administration and demonstrate a low order of acute toxicity.

4.3.1.2.1 Acute Oral Toxicity

Commercial oil-based samples of eight of the twelve substances in the zinc dialkyldithiophosphate category have been tested for acute oral toxicity. The acute oral LD_{50} for these studies in rats ranged from 2000-3500 mg/kg. Clinical signs observed following treatment included diarrhea, lethargy, reduced food consumption, and staining about the nose and eye. Ptosis, piloerection, ataxia and salivation were occasionally observed. The incidence and severity of these

symptoms were proportional to the dose. In many cases the effects were found to be reversible during observation week 2. Necropsy findings were few in number. Lung congestion, gastrointestinal irritation and a reduction in body fat were observed in some animals. Significant necropsy findings in survivors were uncommon. Overall, the acute oral LD₅₀ for these substances ranged from 2000 – 3500 mg/kg indicative of a relatively low order of lethal toxicity.

4.3.1.2.2 Acute Dermal Toxicity

Commercial oil-based samples for nine of the twelve substances in the zinc dialkyldithiophosphate category have been tested for acute dermal toxicity. The acute dermal LD₅₀s for these studies in rabbits were greater than 2000 mg/kg (limit tests). No treatment-related mortality was observed at doses ranging from 2000-8000 mg/kg. Dermal application of the test materials to abraded skin for 24 hours typically produced moderate-to-severe erythema and edema, which in some cases persisted through the 14-day observation period. Clinical signs included varying degrees of reduced food consumption, weight loss, diarrhea, lethargy, ataxia, ptosis, motor incoordination and/or loss of righting reflex. There were no remarkable gross necropsy observations. Overall, the acute dermal LD₅₀ for these substances were greater than 2000 mg/kg indicative of a relatively low order of lethal toxicity.

4.3.1.3 Data Assessment and Test Plan for Acute Mammalian Toxicity

In total, seventeen adequate acute toxicity studies have been conducted commercial samples of the zinc dialkyldithiophosphate in highly refined lubricating base oil. These studies involved two species of laboratory animals (rats or rabbits); two routes of exposure (oral and dermal); and evaluated the toxicity of ten of the twelve members of the category. The substances tested ranged from those with the shortest (C3-C6) alkyl side chains (mixed isobutyl and pentyl derivative; and mixed isopropyl and 1,3-dimethylbutyl derivative) to the derivative containing diisodecyl (C10) esters. The data consistently demonstrate a low order of acute toxicity regardless of the length of the alkyl side chain. The overall low order of acute toxicity for these substances in combination with their similar chemical structure and physicochemical properties supports the scientific justification of these twelve substances as a category within the HPV Challenge Program.

Bridging will be used to fill the acute toxicity data gaps for the remaining four category members.

Acute toxicity data for the mixed isopropyl and 1,3-dimethylbutyl derivative, mixed isobutyl and pentyl derivative, mixed sec-butyl and 1,3-dimethylbutyl derivative, mixed isobutyl and 2-ethylhexyl derivative can be bridged to the following category members:

- Mixed sec-butyl and isooctyl derivative

- Mixed isobutyl, pentyl, and isooctyl derivatives.

This bridging is justifiable based on the increasing length of the alkyl side chains in this range of the category (from mixed C3-C6 to mixed C4-C8) and the lack of an increasing trend in acute toxicity across the entire category.

Acute toxicity data for the 1,3-dimethylbutyl derivative, 2-ethylhexyl derivative, isooctyl derivative can be bridged to:

- diisodecyl derivative.

This bridging is also justifiable based on the increasing length of the alkyl side chains in this range of the category (from C6 to C8) and the lack of an increasing trend in acute toxicity across the entire category.

Acute toxicity data for the tetrapropenyl derivative will be bridged to:

- dodecylphenol derivative since both members of the category are characterized by C12 alkyl side chains on an aromatic ring.

By bridging these data to the four untested substances, the acute toxicity of the category has been evaluated with respect to all acute toxicity endpoints, and no additional acute toxicity testing is proposed for the HPV Challenge Program.

4.3.2 Mutagenicity of the Zinc Dialkyldithiophosphate Category

4.3.2.1 Mutagenicity Test Methodology

Genetic toxicology is concerned with the effects of substances on genetic material (i.e., DNA and chromosomes). Within genetic material, the gene is the simplest functional unit composed of DNA. Mutations are generally non-lethal, heritable changes to genes that may arise spontaneously or because of xenobiotic exposure. Genetic mutations are commonly measured in bacterial and mammalian cells. The simplest test systems measure the occurrence of a base-pair substitution mutation in which a single nucleotide is changed followed by a subsequent change in the complementary nucleotide on the other DNA strand. Frame shift mutations occur following the deletion or insertion of one or more nucleotides, which then changes the “reading frame” for the remainder of the gene or multiple genes. Genetic testing for these types of point mutations is generally accomplished by *in vitro* cellular assays for forward or reverse mutations. A forward mutation occurs when there is a detectable change in native DNA whereas a reverse mutation occurs when a mutated cell is returned to its initial phenotype. Both base-pair substitutions and frame shift mutations are routinely measured in bacterial cells by measuring the ability of a cell to acquire the capability to grow in an environment missing an essential amino acid. In these tests, a large number of cells are examined to demonstrate a significant increase in the frequencies of mutations that occur over the frequency of spontaneous mutations.

Chromosomal aberrations are large-scale numerical or structural alterations in eukaryotic chromosomes including deletions (visualized as breaks), translocations (exchanges), non-disjunction (aneuploidy), and mitotic recombination. Chromosomal breakage is the classical end point in chromosomal aberration assays. Substances that induce structural changes in chromosomes, especially chromosome breaks, are referred to as "clastogens." To visualize chromosomes and chromosomal aberrations following *in vitro* or *in vivo* treatment with a substance, cells are arrested in metaphase, treated to swell the chromosomes, fixed, transferred to slides and stained. The first metaphase following treatment is the time at which the greatest number of cells with damaged chromosomes may be observed. The most frequently used test systems investigate changes in mammalian cells (such as Chinese hamster ovary or lung cells; human or rat lymphocytes; or human, rat or mouse bone marrow cells) following either *in vitro* or *in vivo* exposure to the test substance. The micronucleus test is a common *in vivo* assay that measures the frequency of micronuclei formation (i.e., chromosomal fragments) in polychromatic erythrocytes.

4.3.2.2 Summary of Mutagenicity Data

A summary of the mutagenicity information for commercial samples of zinc dialkyldithiophosphates in highly refined lubricating base oil category is presented in Table 7. *In vitro* bacterial gene mutation assays, *in vitro* mammalian gene mutation assays, or *in vivo* chromosomal aberration assays have been conducted for seven of the twelve members of the category. Frequencies of reverse mutations in bacteria were not significantly changed after exposure to the zinc dialkyldithiophosphates. *In vitro* mutation studies in mammalian cells indicate that the zinc dialkyldithiophosphates do not consistently display mutagenic activity in the absence of metabolic activation, however, upon biotransformation, these materials showed mutagenic activity. The findings in bacterial and mammalian cells did not vary in proportion to the alkyl chain length or any other physicochemical parameter.

4.3.2.2.1 Bacterial Gene Mutation Assay

Commercial oil-based samples of four of the twelve substances in this category have been tested in a bacterial reverse mutation test (OECD Guidelines 471 and/or 472). All tested substances were negative for mutagenic activity, with and without metabolic activation.

4.3.2.2.2 Mammalian Gene Mutation Assay in Non-transformed Cells

Commercial oil-based samples of six of the twelve substances in this category were tested in an *in vitro* point mutation assay in mouse embryo cells

(Schechtman and Kouri, 1977¹⁰). The results of the studies performed in the absence of hepatic microsome activation were inconsistent, but in general indicating that zinc dialkyldithiophosphates have mutagenic potential (3 studies negative, 3 studies positive in the absence of metabolic activation). However, the weight of evidence (2 studies positive, 1 study negative) indicates that metabolic activation of zinc dialkyldithiophosphates by induced hepatic microsomal enzymes results in a significant increase in the mutagenic potential of this class of chemical substances.

4.3.2.2.3 Mammalian Gene Mutation Assay in Transformed Cells

Commercial oil-based samples of five of the twelve substances in this category were tested in an *in vitro* mouse lymphoma cell mutagenicity assay (Guideline 476, *In vitro Mammalian Cell Gene Mutation Test*). The results of these studies indicate that, in the absence of hepatic microsome activation, zinc dialkyldithiophosphates are not mutagenic. However, the weight of evidence indicates that metabolic activation of zinc dialkyldithiophosphates by induced hepatic microsomal enzymes results in a significant increase in the mutagenic potential of this class of chemical substances.

4.3.2.2.4 *In vivo* Chromosomal Aberration Assays

Commercial oil-based samples of four of the twelve substances in this category were tested in an *in vivo* chromosomal aberration assay (OECD Guideline 474, *Mammalian Erythrocyte Micronucleus Test*). All test substances were negative for clastogenicity.

4.3.2.3 Data Assessment and Test Plan for Mutagenicity

Commercial samples for seven of the twelve zinc dialkyldithiophosphates in highly refined lubricating base oil category have been evaluated for genotoxic potential in tests for gene mutations and chromosomal aberrations. The assays included point mutations in bacteria, two types of cultured mammalian cells, and *in vivo* chromosomal aberrations in mice. The findings from the bacterial reverse mutation assay and *in vivo* mouse micronucleus tests were negative for mutagenic potential for all of the tested materials with alkyl side chains that ranged from mixed C3-C6 to C8. The results from the *in vitro* mammalian cell gene mutation test gave inconsistent results for four members of the category with alkyl side chains that ranged from mixed C4-C8. The results from the *in vitro* BALB/3T3 point mutation (ouabain locus) and cell transformation assays indicate that zinc dialkyldithiophosphates may have genotoxic potential. Despite the high cytotoxicity, variability and mixed test results, the overall data indicated that microsome-activated zinc dialkyldithiophosphates were mutagenic. However,

¹⁰ Schechtman LM and Kouri RE. (1977) Control of benzo(a)pyrene-induced mammalian cell cytotoxicity, mutagenicity and transformation by exogenous enzyme fractions. In: Progress in Genetic Toxicology, D. Scott, B.A. Bridges and F.J. Sobels, eds. Elsevier/North-Holland Biomedical Press, New York, pp. 307-316.

zinc ion has been shown to cause cytotoxicity and mutagenicity in similar cultured mammalian cell systems (Amaker et al., 1979¹¹). Therefore, as a follow-up to this report, two materials containing zinc (zinc chloride and zinc oleate) were tested in the BALB 3T3 point mutation and cell transformation assays. These two zinc salts were found to be mutagenic in these systems. Further, no mutagenic activity was attributed to a calcium analog of a zinc dialkyldithiophosphate that had previously shown activity in these *in vitro* mammalian cell assays. These findings point to zinc ion as the causative subcomponent in the *in vitro* mammalian cell studies. However, genotoxicity studies conducted in a variety of test systems have failed to provide unequivocal evidence for mutagenicity of zinc (ATSDR, 1992¹²). Furthermore, the US Food and Drug Administration (1982¹³) concluded that zinc ion is not carcinogenic. Dermal carcinogenicity tests conducted in mice revealed that new motor oils containing between 1% and 3% zinc dialkyldithiophosphate were found to be non-carcinogenic (American Petroleum Institute). In summary, the weight of evidence supports the conclusion that zinc dialkyldithiophosphates have a low potential genotoxicity, and that these substances do not present a significant risk for mutagenicity or carcinogenicity in humans.

Bridging will be used to fill the genetic toxicity data gaps for the remaining twelve substances.

Bacterial gene mutation and *in vivo* chromosomal aberration data for commercial samples of the mixed isopropyl and 1,3-dimethylbutyl derivative in highly refined lubricating base oil will be bridged to:

- mixed isobutyl and pentyl derivative.

Bacterial gene mutation and *in vivo* chromosomal aberration data for commercial oil-based samples of the mixed sec-butyl and 1,3-dimethylbutyl derivative and the mixed isobutyl and 2-ethylhexyl derivative will be bridged to:

- mixed sec-butyl and isooctyl derivative
- mixed isobutyl, pentyl, and isooctyl derivative.

Bacterial gene mutation and *in vivo* chromosomal aberration data for a commercial sample of the 2-ethylhexyl derivative in highly refined lubricating base oil will be bridged the remaining members of the category with longest alkyl chain lengths:

- Isooctyl derivative
- Diisodecyl derivative
- Dodecyl phenol derivative

¹¹ Amacher et al. Mammalian Cell Mutagenesis: Maturation of Test Systems. Banbury Report 2, 277-293, 1977

¹² Toxicological Profile for Zinc. Prepared by Syracuse Research Corporation and Clement International Corporation for the United States Department of Health and Human Service, Public Health Service, Agency for Toxic Substances and Disease Registry, October 1992

¹³ 47 FR 47441 October 1982, corrected 48 FR 3381 January 1983

- Tetrapropenyl phenol derivative

By bridging these data to those substances which lack bacterial gene mutation and *in vivo* chromosomal aberration data, the genetic toxicity of the category has been evaluated with respect to all mutagenic and clastogenic endpoints, and no additional genetic toxicity testing is proposed for the HPV Challenge Program.

4.3.3 Repeated-dose Toxicity of the Zinc Dialkyldithiophosphate Category

4.3.3.1 Repeated-dose Toxicity Test Methodology

Repeated-dose toxicity studies evaluate the systemic effects of repeated exposure to a chemical over a significant period of the life span of an animal (rats, rabbits, or mice). Chronic repeated-dose toxicity studies are concerned with potential adverse effects upon exposure over the greater part of an organism's life span (e.g., one to two years in rodents). Subchronic repeated-dose studies are also concerned with effects caused by exposure for an extended period, but not one that constitutes a significant portion of the expected life span. Subchronic studies are useful in identifying target organ(s), and they can be used in selecting dose levels for longer-term studies. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of at least 28 days or up to 90 days (i.e., 4 to 13 weeks). A recovery period of two to four weeks (generally included in most study designs) following completion of the dosing or exposure period provides information on whether or not the effects seen during the exposure period are reversible upon cessation of treatment. The dose levels evaluated in repeated-dose toxicity studies are notably lower than the relatively high limit doses used in acute toxicity studies. The NOAEL (no observed adverse effect level), usually expressed in mg/kg/day, defines the dose of test material that produces no significant toxicological effects. If the test material produces toxicity at the lowest dose tested (i.e., there is no defined NOAEL), the lowest dose that produced an adverse effect is defined as the LOAEL (lowest observed adverse effect level). While these studies are designed to assess systemic toxicity, the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

Reproductive and developmental toxicity studies generate information on the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, and development of the conceptus, parturition, and post-partum development of the offspring. Various study designs exist, but they all involve exposure to both male and female test animals before mating. The rat is most often selected as the test species. The test substance is administered to males and females continuously at several graduated doses for at least two weeks prior to mating and until the animals are sacrificed. The males are treated for at least two more weeks. Male gonadal histopathology is carefully assessed at the end of the study. The females are treated through

parturition and early lactation. The adult females and offspring are typically studied until termination on post-natal day 21, or sometimes earlier. In addition to providing data on fertility and reproduction, this study design provides information on potential developmental toxicity following prenatal and limited post-natal exposure to the test substance. A NOAEL or LOAEL is also used to describe the results of these tests, with the exception that these values are derived from effects specific to reproduction or development.

4.3.3.2 Summary of Repeated-Dose Toxicity Data

A summary of the results from the repeated-dose studies for commercial samples of zinc dialkyldithiophosphates in highly refined lubricating base oil is presented in Table 8. Repeated-dose toxicity tests have been performed on six members of the zinc dialkyldithiophosphate category by two routes of administration and in two species of laboratory animals.

4.3.3.2.1 Systemic Toxicity Tests

Six of the 12 substances in the zinc dialkyldithiophosphate category have been tested for subchronic toxicity.

Commercial oil-based samples of 1,3-dimethyl butyl derivative (CAS # 2215-35-2), mixed sec-butyl and isooctyl derivative (CAS # 113706-15-3), mixed isobutyl and 2-ethylhexyl derivative (CAS # 26566-95-0), mixed isobutyl, pentyl and isooctyl derivative (CAS # 68988-46-5), and 2-ethylhexyl derivative (CAS # 4295-15-8) were each evaluated in separate 21-28 day repeated-dose dermal toxicity studies in rabbits (methodologies consistent with OECD Guideline 410, *Repeated Dose Dermal Toxicity: 21/28 Day*). The concentration of test articles applied to the skin in these studies ranged from 3-100%. Deaths were common at the higher concentrations but the incidence decreased in proportion to a reduction in dose. The clinical signs throughout the treated groups included ano-genital staining, nasal and ocular bloody discharge, lacrimation, diarrhea, lethargy, anorexia, adipsia, loss of body weight, emaciation, and behavioral distress. Moderate-to-severe dermatitis (erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation) at the site of topical application was observed in all the treated animals and to a lesser degree in control animals exposed to vehicle. The incidence and severity was proportional to the concentration and duration of exposure to the test material. Significant reductions in hemoglobin, hematocrit and erythrocyte counts were noted in test material treated groups. In addition, several clinical chemistry parameters (alkaline phosphatase, BUN, bilirubin, albumin and cholesterol) were affected by treatment with the test material. Testes and epididymal weights were markedly reduced in the high dose groups and to a lesser degree after lower doses. Adrenal and kidney weights were elevated in some higher dose groups. Microscopic examination of the testes revealed aspermatogenesis, diffuse tubular hypoplasia and reduced

mitotic activity. In no dermal study was a NOAEL for systemic toxicity established.

The 2-ethylhexyl derivative (CAS # 4295-15-8) in highly refined lubricating base oil was evaluated in a 28-day repeated dose oral toxicity study in rats (OECD Guideline 407, *Repeated Dose 28-Day Oral Toxicity Study in Rodents*). The test material was administered to rats by oral gavage at 10, 50, 125, 250 and 500 mg/kg/day for 28 consecutive days. Three animals of each sex died at the high dose. One female died at 125 mg/kg/day. Clinically significant findings related to the test material included rales, salivation, and reductions in body weight gain. Necropsy findings included thickened mucosa of the non-glandular mucosa of the stomach in the mid and high dose animals accompanied by microscopic evidence of submucosal edema and suppurative inflammation. Adrenal weights were increased in the high dose animals without evidence of histopathologic abnormalities. The NOAEL was established at 10 mg/kg/day.

4.3.3.2.2 Reproduction and Developmental Toxicity Tests

The 2-ethylhexyl derivative (CAS # 4295-15-8) in highly refined lubricating base oil was tested for reproduction and developmental toxicity (OECD Guideline 421 *Reproduction/ Developmental Screening Test*). The test material was administered to rats by oral gavage at doses of 30, 100 and 200 mg/kg/day. Male and female rats in each dose group received daily treatment for 14 days prior to, and during, the mating period. In addition, the females were treated during gestation and through day 4 of lactation. Control animals received corn oil. Results. Treatment-related deaths (2/12 males, 3/12 females) were recorded in the high dose group. Clinical signs in the decedents included respiratory distress, salivation, hunched posture and mucoid diarrhea. At necropsy, gastric irritation was also observed in the decedents. Mean body weight gain was found to be significantly reduced in the high dose group only. Apart from the gastric observations in the high dose decedents, there were no significant organ weight or microscopic changes because of treatment. There were no significant treatment related effects on reproductive indices or microscopic anatomy of the reproductive organs in the parents of any group. Pup viability in the mid and high dose groups was reduced at parturition and in the post-natal period. The toxicological significance of this finding was questionable. No treatment related effects were observed upon necropsy of the pups found dead or at the scheduled termination. The NOAEL was determined to be 30 mg/kg/day for the parental animals (mortality, clinical signs) and 30 mg/kg/day for the F1 offspring (neonatal mortality).

4.3.3.3 Data Assessment and Test Plan for Repeated-dose Toxicity

Adequate data for repeated-dose toxicity exist for commercial oil-based samples of six of the twelve substances in the zinc dialkyldithiophosphate category. The results of these studies indicate that in repeated dermal exposure to these materials in rabbits can cause moderate-to-severe dermatitis, significant loss of body weight, behavioral distress, reductions in hematology parameters, loss of normal testicular function, and even death at the higher doses. These effects were observed across several members of the category with carbon chain lengths ranging from C4-C8. There was no evidence that the incremental increase in carbon chain length or molecular weight could be correlated with significant changes in toxicity parameters.

Repeated oral administration of commercial samples of zinc dialkyldithiophosphates in highly refined lubricating base oil resulted in evidence of severe gastrointestinal irritation with submucosal edema and suppurative inflammation, mucoid diarrhea, significant body weight loss, distress, and death at the higher doses. However, no significant adverse effects were noted on the testes or male accessory reproductive organs.

The totality of the repeated dose toxicity data indicates that the reproductive organ effects observed in male rabbits are attributed to the stress associated with the severe dermal responses to the test material, rather than direct a systemic response to the test materials. Changes in male reproductive organs in the rabbit have been observed when other irritating substances are applied to the skin at dose levels that cause skin lesions.^{14,15} Thus, dermal irritation alone, or in combination with the accompanying weight loss and stress, is thought to play a role in the reproductive organ response to repeated cutaneous application of zinc dialkyldithiophosphates.

4.3.3.4 Repeated-dose toxicity bridging arguments

- Data for the six zinc dialkyldithiophosphates in highly refined lubricating base oil with carbon chain lengths ranging from C4-C8 indicate similar effects, largely attributable to profound dermal irritation at the site of test material application. There was no evidence of increasing toxicity that could be correlated with an increase or decrease in carbon chain length or molecular weight. Consequently, available repeated dose data on the six intermediate carbon chain length zinc dialkyldithiophosphates in highly refined lubricating

¹⁴Wong, Z. A., VonBurg, R., Spangler, W. L., and MacGregor, J. A. (1982) Testicular Damage in the Rabbit Resulting from Simple Chemical Cutaneous Irritation. *The Toxicologist* 2: 41.

¹⁵McKee, R. H., Kapp, Jr., R. W., and Ward, D. P. (1985) Evaluation of the Systemic Toxicity of Coal Liquefaction-Derived Materials Following Repeated Dermal Exposure in the Rabbit. *J. App. Toxicol.* 5: 345-351.

base oil will be used as read across to the following category members of similar or higher carbon chain length (molecular weight):

- Mixed isobutyl and pentyl derivative (CAS # 68457-79-4),
- Mixed sec-butyl and 1,3-dimethylbutyl derivative (CAS # 68784-31-6),
- Diisodecyl derivative (CAS # 25103-54-2),
- Dodecylphenol derivative (CAS # 54261-67-5), and
- Tetrapropenylphenol derivative (CAS # 11059-65-7)

4.3.3.5 Repeated-dose reproduction/developmental toxicity bridging arguments

- Reproduction/developmental toxicity data was available for a commercial sample of the 2-ethylhexyl derivative in highly refined lubricating base oil. Although the repeated dose dermal toxicity studies indicated that exposure to zinc dialkyldithiophosphates can have adverse effects on testicular function, it is believed that these effects are secondary to profound dermal irritation, body weight loss and stress. This is evidenced by the lack of reproductive organ effects in the oral reproduction/developmental toxicity assay observed with the 2-ethylhexyl derivative and supportive literature linking dermal irritation and testicular function in experimental animals. Furthermore, epidemiological studies support the lack reproductive findings in experimental analyses. In 1980, National Institute for Occupational Safety and Health investigators conducted a survey to obtain process and toxicology research information, and to conduct employee interviews at a zinc dialkyldithiophosphate production plant¹⁶. The plant produced zinc dialkyldithiophosphates of various chain lengths ranging from C3-C8. Review of medical histories showed no significant difference between the “exposed” and “controls” zinc dialkyldithiophosphate manufacturing plant workers with regard to birth defects in offspring, or infertility, miscarriages and stillbirths experienced by wives or partners. Physical examination showed no gross abnormalities in secondary sexual characteristics for exposed and controls. Semen analysis showed no azoospermia or oligospermia in the exposed group. Other parameters of the semen analysis showed no significant difference between exposed and controls. The conclusion of the report was that workers exposed to zinc dialkyldithiophosphates in an occupational setting did not exhibit untoward effects on reproductive health when compared to workers not exposed to such compounds.

Given this information, it is reasonable that the results on the epidemiological study on workers in a zinc dialkyldithiophosphate (C3-C8) manufacturing plant in combination with reproduction/developmental toxicity study results on the 2-ethylhexyl derivative (CAS # 4295-15-8) be used as read-across to the following members of the category:

¹⁶ NIOSH Health Hazard Evaluation Report HETA 80-228-1241, 1980.

- Mixed isopropyl and 1,3-dimethylbutyl derivative (CAS # 84605-29-8)
- Mixed isobutyl and pentyl derivative (CAS # 68457-79-4)
- Mixed sec-butyl and 1,3-dimethylbutyl derivative (CAS # 68784-31-6)
- Mixed sec-butyl and isooctyl derivative (CAS # 113706-15-3)
- Mixed isobutyl and 2-ethylhexyl derivative (CAS # 26566-95-0)
- 1,3-dimethylbutyl derivative (CAS # 2215-35-2)
- Mixed isobutyl, pentyl and isooctyl derivative (CAS # 68988-46-5)
- Isooctyl derivative (CAS # 28629-66-5)
- Diisodecyl derivative (CAS # 25103-54-2),
- Dodecylphenol derivative (CAS # 54261-67-5), and
- Tetrapropenylphenol derivative (CAS # 11059-65-7).

The process of analyzing the existing zinc dialkyldithiophosphate data was performed in a thoughtful and qualitative manner. HERTG concludes that given the relatively minor structural variation between adjacent members of the category, in combination with the totality of human experience and experimental evidence that the reproduction/developmental hazard profile is sufficiently described for the entire category. Therefore, no additional repeated dose toxicity testing is proposed.

The proposal for no additional repeated-dose testing is based on the following considerations.

- 1) Through the collaborative efforts of the lubricant additive manufacturers, zinc dialkyldithiophosphates are perhaps the most extensively reviewed and tested additive component from a health and safety perspective. The industry has several decades of zinc dialkyldithiophosphate use in automotive crankcases without any evidence of repeated dose or cumulative effects on humans.
- 2) As discussed above, there is no evidence of direct effects of repeated doses of zinc dialkyldithiophosphates on reproduction systems or indices. Neonatal mortality in rodents following repeated dosing was observed only in the presence of material toxicity, and thus was considered to be of equivocal toxicological significance. Additional animal testing would not significantly contribute to the understanding of the effect of repeated dose exposure to humans, and is unlikely to demonstrate any additional risk to those in the workplace or to the general public.
- 3) The low potential for repeated dose toxicology in humans is due, in part, to the physical-chemical characteristics of these materials. Zinc dialkyldithiophosphates are high molecular weight components (average > 500 gm/mol), which generally accepted that the molecular weight limit for passive transport across biological membranes. Thus, upon exposure it is unlikely that significant amounts of these components will be absorbed for

systemic distribution. In addition, these materials have a low water solubility that further inhibits absorption and distribution in the mammalian system. A Japanese MITI publication¹⁷ cited a bioaccumulation factor of less than 100 for a C4-C5 ester zinc dithiophosphate indicating a low potential for bioconcentration or cumulative effects. Finally, the negligible vapor pressure and high viscosity at ambient temperature indicates that these materials are unlikely to represent an inhalation exposure under conditions of use.

The exposure profile of zinc dialkyldithiophosphate also demonstrates that there is minimal risk for repeated dose toxicity. Zinc dialkyldithio-phosphates have a singular use in automotive crankcase lubricant additive. Apart from the filling operation, zinc dialkyldithiophosphates are retained in a closed system (i.e., the crankcase), and their use does not result in wide distribution of this component. Furthermore, these materials are designed to undergo thermal decomposition in the crankcase, resulting in the production of a lubricant film on critical engine parts to minimize engine wear and oxidation. Three populations that are most likely to have exposure to zinc dialkyldithiophosphate include manufacture, blending, original equipment manufacturer and downstream automobile service operations. Manufacture of zinc dialkyldithiophosphates is conducted in closed reaction vessels and transfers are performed in closed pipes. The exposure profile to production workers is very low due to process, engineering and personal protection equipment controls. The only practical exposure of concentrated component would be acute and only occur in the rare case of an accidental spill. The dermal route would be the principal means of exposure. Oral or inhalation exposure is expected to be rare.

As mentioned previously, epidemiological studies on workers in zinc dialkyldithiophosphate manufacturing plants did not reveal evidence of cumulative toxicity. Transportation of zinc dialkyldithiophosphates occurs in bulk and transfer to blending operation occurs in closed pipes and vessels. Again, the exposure profile during blending is very low. Zinc dialkyldithiophosphates are then mixed with other lubricant additives, in the presence of high molecular weight, highly refined mineral oil for use as motor oils. The typical concentration of zinc dialkyldithiophosphate in passenger car motor oil is low (e.g., 1-3%). Although OEM factory fill is largely automated and does not result in human contact, dermal exposure to low levels of zinc dialkyldithiophosphate in fresh motor oil is possible for workers in services stations and with do-it-yourself motor oil changers. However, even this small amount of exposure to the general public is falling due to extended drain intervals and the ever increasing fee-for-service lubrication operations staffed by service personnel trained in good occupational hygiene.

¹⁷ Handbook of Existing and New Chemical Substances. Fifth Edition. Edited by the Chemical Products Safety Division, Basic Industries Bureau, Ministry of International Trade and Industry. Published by Japan Chemical Industry Ecology-Toxicology & Information Center. The Chemical Daily Co. 1992.

- 4) In addition to the arguments outlined above, HERTG believes that additional testing of zinc dialkyldithiophosphates will cause unnecessary distress to experimental animals.

Zinc dialkyldithiophosphates are prepared from strong acids that are subsequently neutralized with zinc oxide. Extensive experimental studies demonstrate that zinc dialkyldithiophosphates are skin, eye and mucosal irritants. These hazards are clearly communicated on supplier material safety data sheets (MSDS) and product shipping labels. Animals used in the subchronic dermal toxicity studies were clearly in distress resulting from the severe local skin damage caused by repeated topical administrations of zinc dialkyldithiophosphate. The clinical signs/symptoms along with the supporting gross and microscopic pathology indicate that the experimental animals in the dermal studies experienced distress and suffering. Observations to support this assertion include, but are not limited to, changes in the physical appearance (e.g., blood around eyes and nose as well as ano-genital staining suggestive of a stress- or pain-related condition resulting in secretions not being removed by grooming), and changes in body weight and emaciation (often related changes in food and water consumption due to stress). Furthermore, it is clear that oral administration of zinc dialkyldithiophosphates caused gastrointestinal distress. This conclusion is based on the clinical observations which include salivation and hunched posture following dosing, reductions in body weight, mucoid diarrhea, nasal ocular and ano-genital staining as well as pathological indication of severe gastrointestinal irritation with gastric mucosal edema and suppurative inflammation. It is well accepted that strong mucosal irritants can cause pain, suffering and distress resulting from ulceration and cell death in the stomach lining when administered by the oral route. It is important to remember that the principal hazard of zinc dialkyldithiophosphates is their strong irritant property. HERTG shares EPA's commitment to reduce the number of animals needed for testing and to reduce pain and suffering of test animals to the extent that it is practical and scientifically justifiable. Based on a thoughtful scientific review, HERTG would be unable to conduct additional repeated dose testing of zinc dialkyldithiophosphates without imparting unnecessary and substantial distress and suffering to the experimental animal.

Table 1. Members of the Zinc Dialkyldithiophosphate Category

CAS Number	Chemical Name	Simplified Chemical Name
84605-29-8	Phosphorodithioic acid, mixed O,O-bis(1,3-dimethylbutyl and iso-propyl) esters, zinc salts	Mixed isopropyl and 1,3-dimethylbutyl derivative
68457-79-4	Phosphorodithioic acid, mixed O,O-bis(isobutyl and pentyl) esters, zinc salts	Mixed isobutyl and pentyl derivative
68784-31-6	Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3-dimethylbutyl) esters, zinc salts	Mixed sec-butyl and 1,3-dimethylbutyl derivative
113706-15-3	Phosphorodithioic acid, mixed O,O-bis(sec-butyl and isooctyl) esters, zinc salts	Mixed sec-butyl and isooctyl derivative
26566-95-0	Phosphorodithioic acid, O-(2-ethylhexyl) O-isobutyl ester, zinc salt	Mixed isobutyl and 2-ethylhexyl derivative
68988-46-5	Phosphorodithioic acid, mixed O,O-bis(isobutyl and isooctyl and pentyl) esters, zinc salts	Mixed isobutyl, pentyl and isooctyl derivative
2215-35-2	Phosphorodithioic acid, O,O-bis(1,3-dimethylbutyl) ester, zinc salt	1,3-dimethylbutyl derivative
4259-15-8	Phosphorodithioic acid, O,O-bis(2-ethylhexyl) ester, zinc salt	2-ethylhexyl derivative
28629-66-5	Phosphorodithioic acid, O,O-bis(isooctyl) ester, zinc salt	Isooctyl derivative
25103-54-2	Phosphorodithioic acid, O,O-diisodecyl ester, zinc salt	Diisodecyl derivative
54261-67-5	Phenol, dodecyl-, hydrogen phosphorodithioate, zinc salt	Dodecylphenol derivative
11059-65-7	Phenol, tetrapropenyl-, hydrogen phosphorodithioate, zinc salt	Tetrapropenylphenol derivative

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates

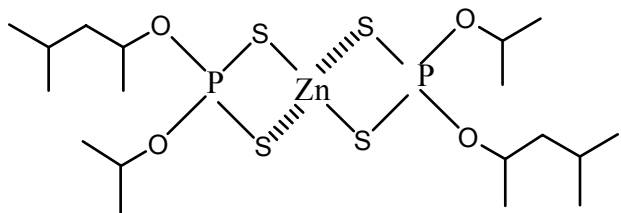
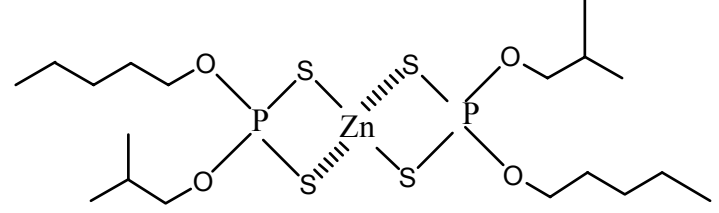
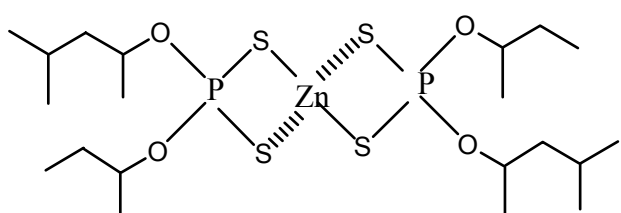
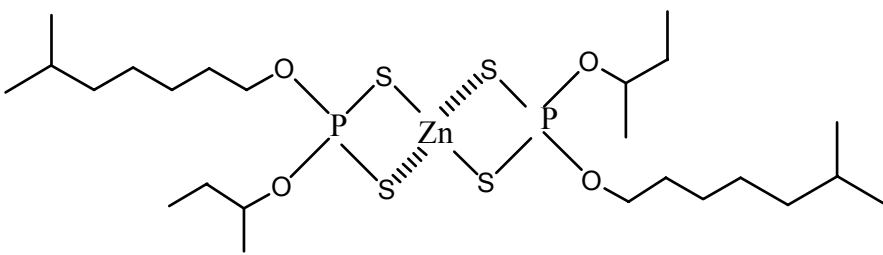
CAS Number	Chemical Structure
84605-29-8	
68457-79-4	
68784-31-6	
113706-15-3	

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates (Cont.)

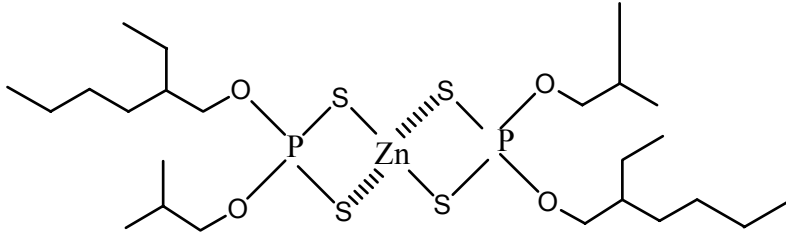
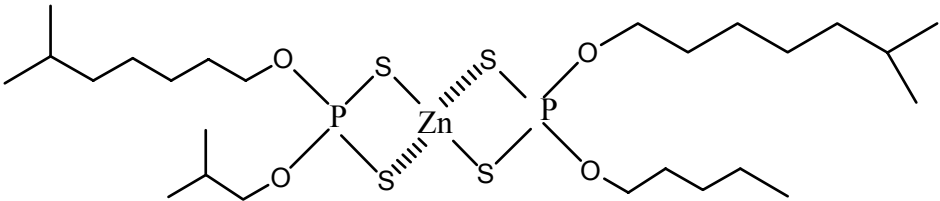
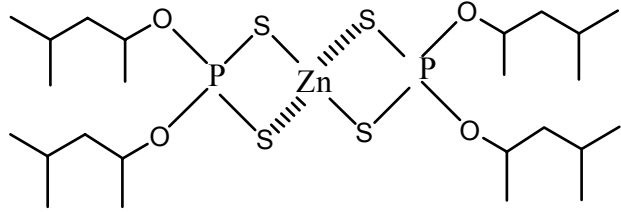
CAS Number	Chemical Structure
26566-95-0	
68988-46-5	
2215-35-2	

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates (Cont.)

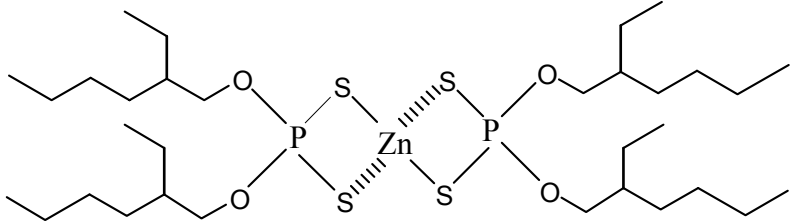
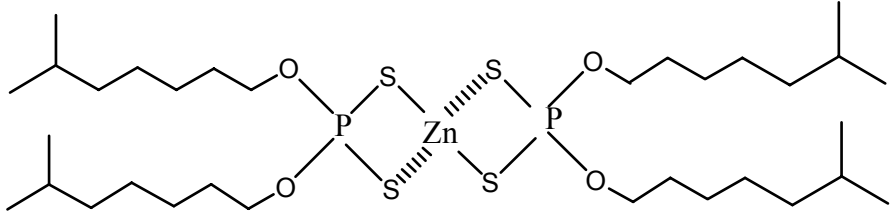
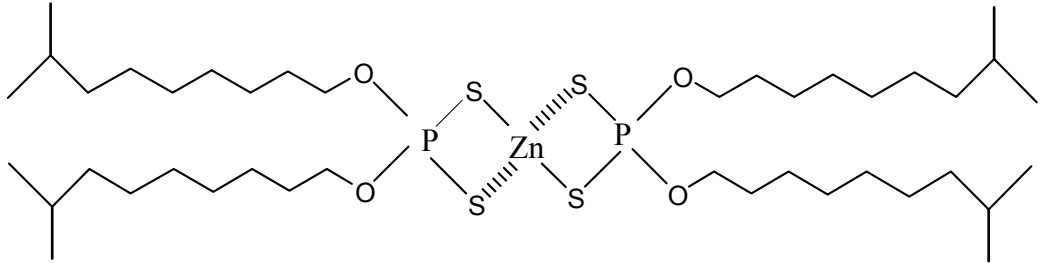
CAS Number	Chemical Structure
4259-15-8	
28629-66-5	
25103-54-2	

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates (Cont.)

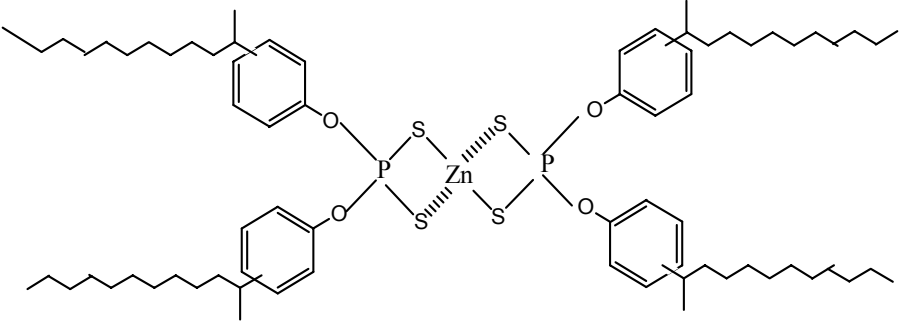
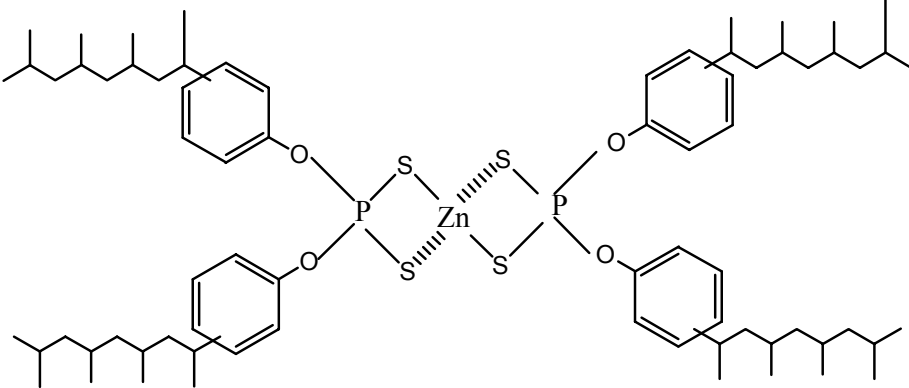
CAS Number	Chemical Structure
54261-67-5	
11059-65-7	

Table 3. Physicochemical Properties of Zinc Dialkyldithiophosphates

CAS Number	Average Molecular Weight (gm/mol)	Alcohol Carbon Number Range	Specific Gravity ² (gm/ml)	Viscosity ³ (cSt)	Melting Point °C	Boiling Point °C	Vapor Pressure ⁴ (mmHg)	Water Solubility (mg/L)
84605-29-8	578.1	C12-C24	1.145	11.0 @ 100°C	NA	Decomp. @ 120°C	<0.5	Test
68457-79-4	578.1	C16-C20	1.120	115.0 @ 40°C	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
68784-31-6	606.2	C16-C24	1.080	8.0 @ 100°C	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
113706-15-3	662.3	C16-C32	No data	No data	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
26566-95-0	648.3	C16-C32	1.135	12.5 @ 100°C	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
68988-46-5	634.2	C16-C32	No data	No data	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
2215-35-2	662.3	C24	No data	No data	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
4259-15-8	774.5	C32	1.099	15.0 @ 100°C	NA	Decomp. @ 120°C	<0.5	Test
28629-66-5	774.5	C32	No data	No data	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
25103-54-2	886.7	C40	1.015	18.0 @ 100°C	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
54261-67-5	1303.3	C72	0.998	30.0 @ 100°C	NA	Decomp. @ 120°C	<0.5	No testing needed Bridging
11059-65-7	1303.3	C72	No data	No data	NA	Decomp. @ 120°C	<0.5	Test

²ASTM D1298-99, Standard Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method

³ASTM D 445-97, Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (the Calculation of Dynamic Viscosity)

⁴“De-oiled” zinc dialkyldithiophosphates are solid. Vapor pressure measurements conducted on a C8 ester zinc dialkyldithiophosphate as manufactured (90% in highly refined lubricating base oil), and on a sample of lubricating base oil by itself yielded values of <0.5 mm Hg.

NA – Not applicable for liquids at ambient temperature

Table 4. Evaluation of Environmental Fate Information for Zinc Dialkyldithiophosphates

CAS Number	BIODEGRADABILITY	HYDROLYSIS	PHOTODEGRADATION
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
84605-29-8	5.9% biodegraded after 28 days	No testing needed	Test (UV Absorption)
68457-79-4	No testing needed Bridging	No testing needed	No testing needed Bridging
68784-31-6	No testing needed Bridging	No testing needed	No testing needed Bridging
113706-15-3	No testing needed Bridging	No testing needed	No testing needed Bridging
26566-95-0	No testing needed Bridging	No testing needed	No testing needed Bridging
68988-46-5	No testing needed Bridging	No testing needed	No testing needed Bridging
2215-35-2	No testing needed Bridging	No testing needed	No testing needed Bridging
4259-15-8	No testing needed Bridging	No testing needed	No testing needed Bridging
28629-66-5	No testing needed Bridging	No testing needed	Test (UV Absorption)
25103-54-2	No testing needed Bridging	No testing needed	No testing needed Bridging
54261-67-5	5.9% biodegraded after 28 days 4.2% biodegraded after 28 days	No testing needed	No testing needed Bridging
11059-65-7	No testing needed Bridging	No testing needed	No testing needed Bridging

Table 5. Evaluation of Aquatic Toxicology of Zinc Dialkyldithiophosphates

CAS Number	ACUTE TOXICITY TO FISH 96-hr LL ₅₀ (mg/L) ¹	ACUTE TOXICITY TO INVERTEBRATES 48-hr EL ₅₀ (mg/L) ¹	TOXICITY TO ALGAE 96-hr EL ₅₀ (mg/L) ¹
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
84605-29-8	Test	Test	Test
68457-79-4	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
68784-31-6	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
113706-15-3	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
26566-95-0	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
68988-46-5	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
2215-35-2	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
4259-15-8	Test	Test	Test
28629-66-5	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
25103-54-2	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
54261-67-5	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
11059-65-7	Test	Test	Test

¹Toxicity endpoints are expressed as median lethal loading rates (LL₅₀) for fish and median effective loading rates (EL₅₀) for *Daphnia* and algae. The EL/LL₅₀ is defined as the loading rate that adversely effects 50% of the test organisms exposed to it during a specific time. The greater the EL/LL₅₀ the lower the toxicity.

F = fathead minnow, *Pimephales promelas*.

D = freshwater cladoceran, *Daphnia magna*.

P = freshwater algae *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*.

T = rainbow trout, *Oncorhynchus mykiss* formerly called *Salmo gairdneri*.

S = sheepshead minnow, *Cyprinodon variegatus*.

R = algae growth rate.

B = algae biomass.

Table 6. Evaluation of Acute Mammalian Toxicology of Zinc Dialkyldithiophosphates

CAS Number	ACUTE ORAL TOXICITY ¹	ACUTE DERMAL TOXICITY ¹
	Available Data & Proposed Testing	Available Data & Proposed Testing
84605-29-8	LD ₅₀ > 2.0 g/kg (rat)	LD ₅₀ > 2.0 g/kg (rabbit)
68457-79-4	LD ₅₀ > 2.0 g/kg (rat)	LD ₅₀ > 5.0 g/kg (rabbit)
68784-31-6	LD ₅₀ > 2.0 g/kg (rat)	LD ₅₀ > 5.0 g/kg (rabbit)
113706-15-3	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
26566-95-0	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
68988-46-5	No testing needed Bridging	LD ₅₀ > 2.0 g/kg (rabbit)
2215-35-2	LD ₅₀ > 2.0 g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
4259-15-8	LD ₅₀ > 2.0 g/kg (rat)	LD ₅₀ > 5.0 g/kg (rabbit)
28629-66-5	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
25103-54-2	No testing needed Bridging	LD ₅₀ > 8.0 g/kg (rabbit)
54261-67-5	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
11059-65-7	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results

¹Toxicity endpoints are expressed as median lethal dose (LD₅₀) for acute oral and dermal toxicity.

Table 7. Evaluation of Mutagenicity of Zinc Dialkyldithiophosphates

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	Available Data & Proposed Testing
84605-29-8	<ul style="list-style-type: none"> Bacterial Reverse Mutation Assay – Not mutagenic <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Not mutagenic in the absence of metabolic activation <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the presence of metabolic activation 	Mouse Micronucleus Assay – Not clastogenic
68457-79-4	<ul style="list-style-type: none"> <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the absence of metabolic activation <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation 	No testing needed Bridging
68784-31-6	Bacterial Reverse Mutation Assay – Not mutagenic	Mouse Micronucleus Assay – Not clastogenic
113706-15-3	<ul style="list-style-type: none"> <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the absence of metabolic activation (only at extremely high toxic doses) <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Mutagenic in the presence of metabolic activation 	No testing needed Bridging

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	Available Data & Proposed Testing
26566-95-0	<ul style="list-style-type: none"> • Bacterial Reverse Mutation Assay – Not mutagenic • <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the absence of metabolic activation • <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the presence of metabolic activation • <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation • <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Equivocal mutagenic response in the presence of metabolic activation 	Mouse Micronucleus Assay – Not clastogenic
68988-46-5	<ul style="list-style-type: none"> • <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Not mutagenic in the absence of metabolic activation • <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation • <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Mutagenic in the presence of metabolic activation 	No testing needed Bridging
2215-35-2	No testing needed Bridging	No testing needed Bridging

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	Available Data & Proposed Testing
4259-15-8	<ul style="list-style-type: none"> • Bacterial Reverse Mutation Assay – Not mutagenic • <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Not mutagenic in the absence of metabolic activation • <i>In vitro</i> Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the presence of metabolic activation • <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation • <i>In vitro</i> Mouse Lymphoma Mutagenicity Assay – Equivocal mutagenic response in the presence of metabolic activation 	Mouse Micronucleus Assay – Not clastogenic
28629-66-5	No testing needed Bridging	No testing needed Bridging
25103-54-2	No testing needed Bridging	No testing needed Bridging
54261-67-5	No testing needed Bridging	No testing needed Bridging
11059-65-7	No testing needed Bridging	No testing needed Bridging

Table 8. Evaluation of Repeated-dose Mammalian Toxicology of Zinc Dialkyldithiophosphates

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
84605-29-8	No testing needed Bridging	No testing needed Bridging
68457-79-4	No testing needed Bridging	No testing needed Bridging
68784-31-6	No testing needed Bridging	No testing needed Bridging
113706-15-3	<p>28-day dermal toxicity study in rabbits NOAEL not established (adverse effects at all doses)</p> <p><u>25%</u></p> <ul style="list-style-type: none"> • Four deaths or moribund sacrifices • Body weight loss • Erythema, edema, atonia, desquamation, eschar formation and exfoliation • Reduction in hemoglobin, hematocrit and erythrocyte counts • Platelet count elevation • Increased serum cholesterol • Decreased serum albumin • Reduction in plasma, erythrocyte and brain cholinesterase levels • Testes and epididymal weight reduction • Adrenal and kidney weight elevation • Morphological abnormalities in the seminiferous tubules characterized by aspermatogenesis, diffuse tubular hypoplasia and reduced mitotic activity <p><u>5%</u></p> <ul style="list-style-type: none"> • One death • Body weight loss • Erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation 	No testing needed Bridging

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
	<ul style="list-style-type: none"> Increased serum cholesterol Reduction in plasma, erythrocyte and brain cholinesterase levels Kidney weight elevation <p><u>Vehicle control</u></p> <ul style="list-style-type: none"> Dermal irritation (lesser degree than in treatment groups) 	
26566-95-0	<p>21-day repeated-dose dermal study in rabbits NOAEL not established (adverse effects at all doses)</p> <p><u>860 mg/kg/day</u></p> <ul style="list-style-type: none"> One death (male) Decedent clinical signs included severe dermal reactions, loss of body weight, anorexia, adipsia, diarrhea, lethargy Survivor clinical signs included moderate-severe dermal reactions, weight loss, nasal and ocular discharge, gastrointestinal distress, occasional lethargy and ptosis, suppression of sperm formation (aspermia) <p><u>430 mg/kg/day</u></p> <ul style="list-style-type: none"> Two deaths (male and female) Decedent clinical signs included severe dermal reactions, loss of body weight, anorexia, adipsia, diarrhea, lethargy Survivor clinical signs included moderate-severe dermal reactions, weight loss, nasal and ocular discharge, gastrointestinal distress, occasional lethargy and ptosis, suppression of sperm formation <p><u>210 mg/kg/day</u></p> <ul style="list-style-type: none"> No deaths Survivor clinical signs included moderate-severe dermal reactions, weight loss, nasal and ocular discharge, 	<p>No testing needed Bridging</p>

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
	<p>gastrointestinal distress, occasional lethargy and ptosis</p> <p><u>Control</u></p> <ul style="list-style-type: none"> • Various signs of distress including nasal and ocular discharge, gastrointestinal distress, occasional lethargy and ptosis, • One animal with severely reduced spermatogenesis 	

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
68988-46-5	<p>21-day repeated-dose dermal study in rabbits NOAEL not established (adverse effects at all doses)</p> <p><u>100%</u></p> <ul style="list-style-type: none"> • All animals in group died (18) • Moderate-severe dermal reactions in proportion to dose • Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge • Severe body weight losses • Reductions in hematology parameters <p><u>25%</u></p> <ul style="list-style-type: none"> • 15/18 animals died • Moderate-severe dermal reactions in proportion to dose • Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge • Severe body weight losses • Reductions in hematology parameters <p><u>5%</u></p> <ul style="list-style-type: none"> • One death • Moderate-severe dermal reactions in proportion to dose • Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge • Body weight losses • Reductions in hematology parameters <p><u>3%</u></p> <ul style="list-style-type: none"> • No deaths • Moderate-severe dermal reactions in proportion to dose • Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge • Body weight losses 	<p>No testing needed Bridging</p>

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
	<ul style="list-style-type: none"> • Reductions in hematology parameters <u>Vehicle control</u> <ul style="list-style-type: none"> • One death • Moderate-severe dermal reactions in proportion to dose • Body weight losses <u>Sham control</u> <ul style="list-style-type: none"> • No deaths 	
2215-35-2	<p>21-day repeated-dose dermal study in rabbits NOAEL not established (adverse effects at all doses)</p> <p><u>1.6 ml/kg/day</u></p> <ul style="list-style-type: none"> • Three animals sacrificed moribund • Severe erythema and edema at the site of application • Body weight loss • White blood cell count reductions • Increased serum triglyceride, uric acid, SGOT, LDH and GGT • Reductions in testes, liver, heart and ovary weights • Decreased spermatogenesis <p><u>0.8 ml/kg/day</u></p> <ul style="list-style-type: none"> • One death • Severe erythema and edema at the site of application • Progressive weight loss over the course of study • White blood cell count reductions <p><u>Naïve control</u></p> <ul style="list-style-type: none"> • Two deaths 	<p>No testing needed Bridging</p>
4259-15-8	<p>28-day repeated-dose oral study in rats (OECD 407) NOAEL = 10 mg/kg/day</p>	<p>Reproduction/developmental oral toxicity screening test in rats (OECD 421) NOAEL P0 = 30 mg/kg/day</p>

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
	<p><u>500 mg/kg/day</u></p> <ul style="list-style-type: none"> • Four deaths (3 male and 1 female) • Clinical signs included changes in fecal consistency and coloration, staining of body surfaces, rales, salivation, aggressive behavior, reduced food consumption, reduced body weight gain • Necropsy findings included edema, suppurative inflammation and thickening of the mucosa of non-glandular stomach; increase in adrenal weights <p><u>250 mg/kg/day</u></p> <ul style="list-style-type: none"> • No deaths • Clinical signs included changes in fecal consistency and coloration, staining of body surfaces, rales, salivation, aggressive behavior, reduced body weight gain • Necropsy findings included thickened mucosa of non-glandular stomach; increase in adrenal weights <p><u>125 mg/kg/day</u></p> <ul style="list-style-type: none"> • One death (female; not treatment related) • Clinical signs included changes in fecal consistency and coloration, staining of body surfaces, rales, salivation and aggressive behavior <p><u>50 mg/kg/day</u></p> <ul style="list-style-type: none"> • No deaths • Rales and salivation <p><u>10 mg/kg/day</u></p> <ul style="list-style-type: none"> • No significant adverse effects 	<p>NOAEL F1 = 30 mg/kg/day</p> <p>Parental animals</p> <p><u>200 mg/kg/day</u></p> <ul style="list-style-type: none"> • Five deaths (two males, three females) • Clinical signs included respiratory distress, hunched appearance and mucoid diarrhea • Reduced pre-mating body weight gain (males) • Necropsy revealed evidence of gastric irritation • No significant effects on reproductive parameters • No microscopic reproductive organ effects <p><u>100 mg/kg/day</u></p> <ul style="list-style-type: none"> • Deaths • Clinical signs included respiratory distress, hunched appearance and mucoid diarrhea • Necropsy revealed evidence of gastric irritation • No significant effects on reproductive parameters • No microscopic reproductive organ effects <p><u>30 mg/kg/day</u></p> <ul style="list-style-type: none"> • No deaths • No significant treatment-related findings • No significant effects on reproductive parameters • No microscopic reproductive organ effects <p>Offspring effects</p> <p><u>200 mg/kg/day</u></p> <ul style="list-style-type: none"> • One incident of total litter loss • Increased pup mortality during post-natal period <p><u>100 mg/kg/day</u></p> <ul style="list-style-type: none"> • Two incidents of total litter loss

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
		<ul style="list-style-type: none"> Increased pup mortality during post-natal period <u>30 mg/kg/day</u> <ul style="list-style-type: none"> No significant adverse effects
28629-66-5	<p>28-day repeated-dose dermal study in rabbits NOEAL not established (adverse effects at all doses)</p> <p><u>25%</u></p> <ul style="list-style-type: none"> 4/20 animals died Moderate-to-severe dermal reactions (proportional to dose) Body weight losses Alterations in hematology and clinical chemistry parameters Testicular hypotrophy and aspermatogenesis <p><u>5%</u></p> <ul style="list-style-type: none"> No deaths Moderate-to-severe dermal reactions (proportional to dose) Body weight losses Alterations in hematology and clinical chemistry parameters Testicular hypotrophy and aspermatogenesis <p><u>Vehicle control</u></p> <ul style="list-style-type: none"> No deaths 	<p>No testing needed Bridging</p>
25103-54-2	No testing needed Bridging	No testing needed Bridging

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
54261-67-5	No testing needed Bridging	No testing needed Bridging
11059-65-7	No testing needed Bridging	No testing needed Bridging

Table 9. Summary Table

CAS Number	Environmental Fate					Ecotoxicity			Human Health Effects				
	Physical Chem	Photodeg	Hydrolysis	Fugacity	Biodeg	Acute Fish Toxicity	Acute Invert Toxicity	Algal Toxicity	Acute Toxicity	Point Mutations	Chrom Effects	Sub-chronic	Repro/Develop
84605-29-8	T ¹	T ²	D	C	A	T	T	T	A	A	A	B	B
68457-79-4	D	B	D	C	B	B	B	B	A	A	B	B	B
68784-31-6	D	B	D	C	B	B	B	B	A	A	A	B	B
113706-15-3	D	B	D	C	B	B	B	B	B	A	B	A	B
26566-95-0	D	B	D	C	B	B	B	B	B	A	A	A	B
68988-46-5	D	B	D	C	B	B	B	B	A	A	B	A	B
2215-35-2	D	B	D	C	B	B	B	B	A	B	B	A	B
4259-15-8	T ¹	B	D	C	B	T	T	T	A	A	A	A	A
28629-66-5	D	T ²	D	C	B	B	B	B	B	B	B	A	B
25103-54-2	D	B	D	C	B	B	B	B	A	B	B	B	B
54261-67-5	D	B	D	C	A	B	B	B	B	B	B	B	B
11059-65-7	T ¹	B	D	C	B	T	T	T	B	B	B	B	B

A Adequate data available
 C Computer modeling proposed
 B Bridging
 D Technical discussion proposed
 T Test
 T¹ Solubility Testing
 T² UV absorption

AR201-14066B

Substance Group: Group 5 - Zinc Dialkyldithiophosphates

Summary Prepared by: Petroleum Additives Panel
Health & Environmental Research Task Group

Date of last update: October 10, 2002

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1.0 Biodegradation

Robust Summary 5-BioDeg-1

<u>Test Substance</u>	
CAS #	54261-67-5
Chemical Name	Phenol, dodecyl-, hydrogen phosphorodithioate zinc salt
Remarks	This substance is referred to as ----- in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 2.0 "Chemical Description of Zinc Alkyl Dithiophosphate Category" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD Method 310B; U.S. EPA Method 796.3260; ASTM D5864-95.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	N
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	<p><u>Inoculum</u>: Soil was collected from a wooded lot to a depth of 20 cm (surface soil was not included). Prior to use, 200 gm (wet weight) of soil was suspended in 2 L of water, allowed to settle for 30 min and filtered through glass wool. Filtrate was aerated until use. Activated sludge from domestic wastewater treatment plant was sieved through a 2 mm screen, aerated for 4 hours and homogenized in a blender. The sludge was allowed to settle for 30 minutes and supernatant was removed for use.</p> <p><u>Concentration of test chemical</u>: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks.</p> <p><u>Temp of incubation</u>: 20 ± 3°C.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume.</p> <p><u>Test Setup</u>: Total volume of liquid in test chambers was 3 L. The biodegradation test was started by bubbling CO₂ free air through the test media at a rate of 50 to 100 mL per minute. The CO₂ generated within each test chamber was trapped as K₂CO₃ in the</p>

	<p>KOH solution and measured using a carbon analyzer.</p> <p><u>Sampling frequency:</u> CO₂ traps were removed for analysis on Days 2, 5, 11, 13, 16, 18, 23, and 29. On day 28, the test was terminated by the acidification of the test chamber to release dissolved CO₂.</p> <p><u>Controls:</u> Blank and positive controls were included; abiotic and toxicity controls were not. Canola oil was used as the reference substance in the positive controls.</p> <p><u>Analytical method:</u> KOH ("trap") solutions were used downstream of the test flasks to trap generated CO₂ as K₂CO₃. The CO₂ produced was measured using a carbon analyzer.</p> <p><u>Method of calculating measured concentrations:</u> N/A</p> <p><u>Other:</u> A preadapted inoculum was used for the biodegradation test. Equal volumes of the activated sludge supernatant and soil filtrate were combined and supplemented with vitamin free casamino acids and 25 mg/L yeast extract. 100 ml of the supplemented inoculum was combined with 900 ml of test medium within each 2-L erlenmeyer flask. The solutions were aerated with CO₂ free air and test substances added incrementally at concentrations equivalent to 4, 8, and 8 mg C/L on days 0, 7, and 11, respectively. On day 14, an equal volume of each culture was combined and composite inoculum screened using glass wool and homogenized in a blender. Standard plate count prior to the 14-day adaptation period was 1.36×10^5 CFU/mL. Standard plate count after 14-day adaptation was 2.62×10^5 CFU/mL.</p>
<u>Results</u>	
Test Substance Degradation, % after time	5.9% after 28 days
Kinetic (for sample, positive and negative controls)	Reference (Canola oil): 92% (2 - 5 day lag period) Test substance: 5.9% (28d; 11 – 16 day lag period)
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
<u>Conclusions</u>	Test substance degraded 5.9% in 28 days. The reference substance, canola oil, degraded 92% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

Robust Summary 5-BioDeg-2

Test Substance	
CAS #	54261-67-5
Chemical Name	Phenol, dodecyl-, hydrogen phosphorodithioate zinc salt
Remarks	This substance is referred to as ----- in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 2.0 "Chemical Description of Zinc Alkyl Dithiophosphate Category" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD 301F
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	<p><u>Inoculum</u>: The supernatant from the homogenized activated sludge was used as inoculum. The sludge was homogenized in a blender at medium speed for approximately 2 minutes and allowed to settle for approximately 30 minutes. The supernatant was used for inoculum preadaptation. Bacterial counts in the inocula were 1×10^5 to 2×10^6 cells/mL.</p> <p><u>Concentration of test chemical</u>: Approximately 100 mg/L of test material was added to the treatment group by direct weight addition.</p> <p><u>Temp of incubation</u>: $20 \pm 1^\circ\text{C}$.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate the dispersion of the test material. The substances were weighed onto a solid carrier (a small Teflon coupon) and introduced into the medium. The reference material was added directly.</p> <p><u>Test Setup</u>: 900 mL of the test media was mixed with 100 mL of the inoculum. The mixture was continuously stirred in a closed flask at a constant temperature for up to 28 days. A BI-1000 electrolytic respirometer system was used for this study. Each reactor flask is equipped with an electrolytic cell assembly, which generates oxygen to replace the amount consumed by the test mixture in the flask.</p> <p><u>Sampling frequency</u>: The oxygen uptake in all flasks were monitored continuously and recorded automatically every 4 hours throughout the test period using the BI Data Acquisition</p>

	<p>software.</p> <p><u>Controls:</u> Yes (blank and positive controls per guideline). Abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control.</p> <p><u>Analytical method:</u> A BI-1000 respirometer was used to continuously monitor O₂ uptake. The O₂ uptake was recorded every 4 hours.</p> <p><u>Method of calculating measured concentrations:</u> N/A</p> <p><u>Other:</u> A preadapted inoculum was used for the biodegradation test. The activated sludge supernatant was combined and supplemented with vitamin free casamino acids and 25 mg/L yeast extract. 100 ml of the supplemented inoculum was combined with 900 ml of test medium within each 2-L erlenmeyer flask. The solutions were aerated with CO₂ free air and test substances added incrementally at concentrations equivalent to 4, 4, and 8 mg C/L on days 0, 7, and 12, respectively. On day 14, each adapted culture was homogenized in a blender and a composite culture prepared by mixing equal volumes of the homogenized cultures.</p>
<u>Results</u>	
Degradation % after time	4.2% after 28 days
Kinetic (for sample, positive and negative controls)	<p>Reference (sodium benzoate) – 82.3% (28d). An average percent biodegradation of 60% was achieved within 3 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14.</p> <p>Test substance – 4.2% (28d)</p>
Breakdown Products (Y/N) If yes describe breakdown products	N
<u>Remarks</u>	
<u>Conclusions</u>	Test material degraded 4.2% in 28 days. The reference substance, sodium benzoate, reached a level of 82.3% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

Robust Summary 5-BioDeg-3

Test Substance	
CAS #	84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	This substance is referred to as ----- in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 2.0 "Chemical Description of Zinc Alkyl Dithiophosphate Category" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD Method 310B; & EEC Method C.4-C (2).
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	<p><u>Inoculum</u>: Activated sludge from domestic waste water treatment plant was sieved through a 2 mm screen, aerated for 4 hours and homogenized in a blender. The sludge was allowed to settle for 30 mins and supernatant was used as the inoculum the same day that it was prepared.</p> <p><u>Concentration of test chemical</u>: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks.</p> <p><u>Temp of incubation</u>: 20 ± 2°C.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume.</p> <p><u>Test Setup</u>: Total volume of liquid in test chambers was 3 L. The biodegradation test was started by bubbling CO₂ free air through the test media at a rate of 50 to 100 mL per minute. The CO₂ generated within each test chamber was trapped as K₂CO₃ in the KOH solution and measured using a carbon analyzer.</p> <p><u>Sampling frequency</u>: CO₂ traps were removed for analysis on Days 1, 4, 8, 11, 14, 19, 21, 25, and 29. The CO₂ trap nearest the chamber was removed. On day 28, the test was terminated by the acidification of the test chamber and aerated overnight to release dissolved CO₂. The trapping solutions closest to the test chambers were analyzed for inorganic carbon.</p>

	<p><u>Controls</u>: Blank and positive controls were included; abiotic and toxicity controls were not. Sodium benzoate was used as the reference substance in the positive controls.</p> <p><u>Analytical method</u>: KOH ("trap") solutions were used downstream of the test flasks to trap generated CO₂ as K₂CO₃. The CO₂ produced was measured using a carbon analyzer.</p> <p><u>Method of calculating measured concentrations</u>: N/A</p> <p><u>Other</u>: n/a</p>
<u>Results</u>	
Test Substance Degradation, % after time	5.9% after 28 days
Kinetic (for sample, positive and negative controls)	Reference (Sodium benzoate): 99.3%. An average percent biodegradation of 60% was achieved within 7 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14. Test substance: 1.5% (28d)
Breakdown Products (Y/N) If yes describe breakdown products	N
<u>Remarks</u>	
<u>Conclusions</u>	Test substance degraded 1.5% in 28 days. The reference substance, canola oil, degraded 99.3% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

2. Toxicity

Category:

2.1 Acute Toxicity

2.1.1 Acute Oral Toxicity

Robust Summary 5-Acute Oral-1

<u>Test Substance</u>	
CAS #	CAS# 2215-35-2
Chemical Name	2-pentanol, 4-methyl-, hydrogen phosphorodithioate, Zn salt
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	EPA FIFRA 81-1 (November 1982)
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rats/Sprague-Dawley strain
Sex	Male and Female
No. of animals/dose	5/sex
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	2.0 and 5.0 g/kg
Dose volume	0.2 and 0.5 ml/100 g
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (over night) male and female rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = Between 2.0 and 5.0 g/kg (males and females)
Remarks	All of the males and females at the 5 g/kg dose level died three to four days after dosing. Two low dose females died (days 1 and 4). All of the low dose males survived. Signs of toxicity at both dose levels included diarrhea, stained and/or ruffled fur and hypoactivity. Necropsy findings observed in the animals that died included gastrointestinal findings; bloody oral and nasal discharge and signs of diarrhea. There were no significant necropsy findings in the animals that survived to study termination.

<u>Conclusions</u>	The test article, when administered as received to male and female Sprague-Dawley rats, had an acute oral LD50 of between 2.0 and 5.0 g/kg (males and females.).
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/12/00 (RTA-042)

Robust Summary 5-Acute Oral-2

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1975
Species/Strain	Rats/ Sprague-Dawley strain
Sex	Male
No. of animals/dose	10
Vehicle	None
Route of administration	Oral (intra gastric)
Dose level	0, 0, 2.2, 3.3, 5.0, and 7.5 g/kg
Dose volume	Not provided
Control group included	Yes
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to ten fasted male rats at each treatment level. Two control groups were included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.1 (1.8-5.1) g/kg (males)
Remarks	Mortality occurred in males dosed at all dose levels. Two of ten animals died at the low dose level. All animals died at the high dose. Signs of toxicity observed in all groups included diarrhea, depression, and reduced food consumption. Surviving animals exhibited recovery from these signs of toxicity between 3 and 12 days after dosing. At necropsy all treated animals exhibited a reduced amount of body fat. No other treatment related necropsy findings were observed.
<u>Conclusions</u>	The test article, when administered as received to male Sprague-Dawley rats, had an acute oral LD50 of 3.1 (1.8-5.1) g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that this is a summary report.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/31/00 (RTA-041)

Robust Summary 5-Acute Oral-3

<u>Test Substance</u>	
CAS #	CAS# 68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1979
Species/Strain	Rats/Wistar strain
Sex	Male
No. of animals/dose	10
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	2.0, 3.5, 5.0 and 8.75 g/kg
Dose volume	2-10 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to ten fasted (over night) male rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.6 (2.7-4.8) g/kg (males)
Remarks	All of the 8.75 g/kg animals died during the first two days of study. Eight of ten males treated at 5.0 g/kg died during days 1 through 3. Six males at the 3.5 g/kg dose level died between days 2 and 5. No deaths occurred at the 2.0 g/kg dose level. Lethargy, diarrhea, ptosis, chromorhinorrhea, piloerection, and chromodacryorrhea were noted in all groups. These findings were no longer evident in the two lowest groups by the middle of week two. Body weight changes were within expected ranges for the surviving animals. Lung congestion, gastrointestinal findings and staining around the mouth, nose and anus were common necropsy findings for the animals that died.
<u>Conclusions</u>	The test article, when administered as received to male Wistar rats, had an acute oral LD50 of 3.6 g/kg.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/11/00 (RTA-038)

Robust Summary 5-Acute Oral-4

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1981
Species/Strain	Rats/ Sprague-Dawley strain
Sex	Male and Female
No. of animals/dose	5/sex
Vehicle	None
Route of administration	Oral (intra-gastric)
Dose level	0, 1.8, 2.7, 4.0 and 6.0 g/kg
Dose volume	0.4-1.6 ml/kg
Control group included	Yes
Remarks field for test conditions	A single dose of the undiluted test material was administered intra-gastrically to five fasted (over night) male and female rats at each treatment level. A control group was included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.4 (2.2-5.2) g/kg (males); 2.9 (1.9-4.7) g/kg (females)
Remarks	Mortality occurred in males dosed with 4.0 g/kg and greater and in females dosed with 2.7 g/kg and greater. Signs of toxicity included diarrhea, depression, reduced food consumption, weakness, salivation, blood in the urine and death. Body weights in the 2.7 g/kg and greater males and/or females were significantly less than control at seven days. No treatment related necropsy findings were observed.
<u>Conclusions</u>	The test article, when administered as received to male and female Sprague-Dawley rats, had an acute oral LD50 of 3.4 (2.2-5.2) g/kg (males) and 2.9 (1.9-4.7) g/kg (females).
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/11/00 (RTA-040)

Robust Summary 5-Acute Oral-5

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1980
Species/Strain	Rats/ Sprague-Dawley strain
Sex	Male and Female
No. of animals/dose	10/sex
Vehicle	Corn Oil
Route of administration	Oral (intragastric)
Dose level	1500, 1825, 2221, 2702, 3288 and 4000 mg/kg
Dose volume	15 ml/kg
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to ten fasted (over night) male and female rats at each treatment level. (Thirteen females were inadvertently dosed in Group 3 and seven females were dosed in Group 4.) A control group was not included. The animals were observed for signs of toxicity or behavioral changes daily. Individual weights were recorded on the day of dosing, and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
<u>Results</u>	LD50 = 3.2 (2.6-4.0) g/kg (males); 3.1 (2.6-3.8) g/kg (females)
Remarks	Deaths occurred in all groups greater than 1825 mg/kg within 93 hours of dosing. Signs of toxicity included hypokinesia at all dose levels, ataxia at doses above 1825 mg/kg and diarrhea in almost all animals in all dose groups. All surviving animals gained weight. Necropsy findings in animals that died on test were limited to findings suggestive of post mortem changes. No treatment related necropsy findings were observed. Surviving animals had no remarkable necropsy findings.
<u>Conclusions</u>	The test article, when administered as received in corn oil to male and female Sprague-Dawley rats, had an acute oral LD50 of 3.2 (2.6-4.0) g/kg (males); 3.1 (2.6-3.8) g/kg (females).
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/12/00 (RTA-043)

2.1.2 Acute Dermal Toxicity

Robust Summary 5-Acute Dermal-1

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	Similar to OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1975
Species/Strain	Rabbits/New Zealand White
Sex	Male
No. of animals/group	6
Vehicle	None
Route of administration	Dermal
Dose level	0 and 5 g/kg
Dose volume	Not provided.
Control group included	Yes
Remarks field for test conditions	<p>This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that the skin of 3 treated animals were abraded prior to dosing. In addition the guideline calls for the evaluation of males and females using at least one dose level. This study was conducted using males only. These deviations were not considered sufficient to change the outcome of the study.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin of three treated animals was abraded prior to test material administration. A single dose of 5 g/kg of the undiluted test material was administered dermally to six male animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered with an elastic sheet. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. All rabbits were fitted with collars for four days to prevent ingestion of the test material. The animals were observed for 14 days after treatment. Irritation was scored at 24, 48 and 72 hours and at 7 days post treatment. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14. The liver and lungs were examined microscopically.</p>
<u>Results</u>	LD50 > 5.0 g/kg (males)
Remarks	One abraded rabbit died 13 days after dosing. Reduced food consumption was observed in most animals during the last 10 days of

	test. All animals appeared depressed during the last week of study. Muscular weakness was observed in the abraded animals during the last three days of study. Sever erythema and edema were noted in the treated skin at 24 hours post dosing. At seven days the treated skin was thick and escharotic. All treated animals lost weight during the study. Control animals gained weight. At necropsy dermal findings were consistent with the in life evaluation. All animals had reduced amounts of body fat. Microscopic examination of gross lesions observed in the liver and lungs confirmed the presence of bronchopneumonia or chronic interstitial pneumonia and liver parasites. The lungs and livers of the control animals were unremarkable.
<u>Conclusions</u>	The test article, when administered dermally as received to 6 male New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that this is a summary report.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/31/00 (RTA-044)

Robust Summary 5-Acute Dermal-2

<u>Test Substance</u>	
CAS #	CAS# 68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material dosed as received, purity not provided.
<u>Method</u>	
Method/Guideline followed	Similar to OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1975
Species/Strain	Rabbits/New Zealand White
Sex	Male and Female
No. of animals/group	2/sex
Vehicle	None
Route of administration	Dermal
Dose level	20 g/kg
Dose volume	Not provided.
Control group included	No
Remarks field for test conditions	<p>This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that 2 rather than 5 animal/sex were evaluated for toxicity. In addition the skin of one animal/sex was abraded prior to dosing. The guideline does not call for abraded skin. While the guideline calls for gross necropsies to be conducted on all animals they were not conducted during this study. Given the high dose level tested during this study and the lack of any mortality, these deviations were not considered sufficient to disqualify this study.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin of two treated animals was abraded prior to test material administration. A single dose of 20 g/kg of the undiluted test material was administered dermally to two male and two female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered with an elastic sheet. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for 14 days after treatment. Irritation was scored at 24hours post treatment using the Draize method. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were not performed.</p>
<u>Results</u>	
LD50	LD50 > 20.0 g/kg (males and female)
Remarks	All animals survived the study. Well defined erythema and slight edema were noted at 24 hours post dosing. Clinical signs including lethargy, diarrhea, ataxia, ptosis, alopecia, emaciation, nasal discharge

	and sensitivity to touch were noted during the second week of observation. All treated animals lost weight during the study.
<u>Conclusions</u>	The test article, when administered dermally as received to 2 male and 2 female New Zealand white rabbits had an acute dermal LD50 of greater than 20.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that the study design differs significantly from the referenced guideline. However given the high dose level tested (20 g/kg) and the lack of mortality the study was considered valid and appropriate for review.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/31/00 (RTA-045)

Robust Summary 5-Acute Dermal-3

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material purity not provided
<u>Method</u>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1981
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	5 g/kg
Dose volume	5 ml/kg
Control group included	Yes
Remarks field for test conditions	<p>This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline that would adversely effect the study results.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under an elastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. Collars were placed on the animals for six days to prevent ingestion of the test material. The animals were observed for abnormal clinical signs daily for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 7 and 14. Gross necropsies were performed on all animals on Day 14. A section of treated skin was examined from each animal microscopically.</p>
<u>Results</u>	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical signs observed were reduced food consumption and decreased motor activity. Body weights of the treated males and females were significantly less than control on days 7 and 14. Microscopic examination of the treated skin showed diffuse subacute dermatitis. No gross necropsy effects other than skin effects were evident.

<u>Conclusions</u>	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 5.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that most data are not presented in the report. Summary statements are provided.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/13/00 (RTA-049)

Robust Summary 5-Acute Dermal-4

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu,iso-octyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided
<u>Method</u>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1984
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	2 ml/kg
Control group included	No
Remarks field for test conditions	<p>This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze and elastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs frequently on the day of dosing and once daily for 14 days after treatment. Individual body weights were recorded on the day of dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.</p>
<u>Results</u>	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical signs observed in all animals included cyanosis and decreased motor activity. The majority of animals exhibited motor incoordination. Four animals exhibited a loss of righting reflex. Recovery from most of these signs occurred by day three post treatment. Dermal findings included necrosis, edema and ulceration. Dermal irritation persisted through study termination. Gross pathological findings were limited to pitted kidneys in one female.

<u>Conclusions</u>	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/13/00 (RTA-048)

Robust Summary 5-Acute Dermal-5

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	None
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1980
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	2
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	2.2 ml/kg
Control group included	No
Remarks field for test conditions	Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. On the day of dosing the skin was abraded prior to test material administration. A single dose of 2 g/kg of the undiluted test material was administered dermally to two male and two female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze patch and plastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs daily for 14 days after treatment. Individual body weights were recorded on the day of dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.
<u>Results</u>	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed during the study. Erythema was observed in one animal on days 2 and 3 post dosing. All animals exhibited peeling of the skin at the dose site on the last three days of study. One rabbit had pale kidneys at necropsy. All of the remaining animals were unremarkable.
<u>Conclusions</u>	The test article, when administered dermally as received to 2 male and 2 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg. No evidence of systemic toxicity was observed.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/13/00 (RTA-047)

Robust Summary 5-Acute Dermal-6

<u>Test Substance</u>	
CAS #	CAS# 25103-54-2
Chemical Name	isodecanol, hydrogen phosphorodithioate, zinc salt
Remarks	Test material purity not provided
<u>Method</u>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	8 g/kg
Specific Gravity	1.06 g/ml
Control group included	No
Remarks field for test conditions	<p>This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline that would adversely affect the study results.</p> <p>A range finding study, conducted at dose levels of 1, 3.2, 6.3 and 8 g/kg in one animal/sex/dose level preceded this study. Based on the range find study results, a limit test was conducted at the 8 g/kg dose level.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 8 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered by a rubber dam and an elastic bandage. The animals were observed for abnormal clinical signs at 0.5, 2 and 4 hours after the 24-hour period of exposure and twice daily thereafter for 14 days after treatment. Individual body weights were recorded on the day of dosing and on days 7 and 14. Gross necropsies were performed on all animals on Day 14.</p>
<u>Results</u>	LD50 > 8.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical signs observed included moderate erythema and edema, decreased activity, decreased muscle tone, abnormal gait and abnormal stance. Fissuring and sloughing of the skin at the application site were also observed. Terminal necropsy revealed several white nodules throughout the lungs and a discoloration of the abdominal wall in one animal.

<u>Conclusions</u>	The test article, when administered dermally as received to 5 abraded male and 5 abraded female New Zealand white rabbits had an acute dermal LD50 of greater than 8.0 g/kg.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that most data are not presented in the report. Summary statements are provided.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 12/14/01

2.2 Repeated Dose Toxicity

Robust Summary 5-Repeated Tox-1

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	None
Test Type	3 week dermal toxicity study in rabbits
GLP (Y/N)	N
Year (Study Performed)	1979
Species	Rabbit
Strain	New Zealand White
Route of administration	Dermal, 5 days/week, to the clipped, unabraided, dorsal surface.
Duration of test	15 days of treatment
Doses/concentration levels	0, 0.21, 0.43 and 0.86 g/kg
Vehicle control	None
Dose volume	0.2, 0.4, 0.6 mL/kg/day
Sex	Males/Females
Frequency of treatment	Once/day, 5 days/week for a total of 15 doses.
control and treatment groups	5/sex/group
Post exposure observation period	None
Statistical methods	Means and standard deviations were reported
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rabbits 5 days/week for 3 weeks. Six dosing sites were used on each animal. Dosing sites were rotated daily. Elizabethan collars were used to prevent ingestion. Clinical observations were made twice daily. Dermal responses were evaluated daily, 24 hours post treatment (Draize). Body weight was recorded weekly during treatment. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. A range of tissues was examined microscopically in all animals. The testes were examined a second time in 1980.
<u>Results</u>	
Remarks	One high dose male and one mid dose male and female died during the study. These animals exhibited a loss of body weight, lethargy, anorexia, adipsia emaciation and diarrhea prior to death. The surviving animals in the control and all treated groups exhibited various signs of distress including nasal and ocular discharge, and gastrointestinal findings. These findings increased in frequency over the course of the study. They were accompanied, in some cases, by lethargy and ptosis. Several animals exhibited anorexia and adipsia accompanied by emaciation. Dermal reactions were moderate in all of the treated animals through Week 1. Severe reactions were observed in all treated

	<p>animals by the ninth dose. All of the treated males and females at the mid and high dose lost weight during the treatment period. Leukocyte counts were increased slightly in all treated groups at termination. Necropsy findings included sporadic occurrences of dark lungs, broncho-pneumonia, intestinal findings, liver nodules, kidney discolorations and retroperitoneal lymphoma in one rabbit. No clear relationships to treatment were observed. Moderate to severe epithelial hyperplasia with surface exudate was observed in the treated skin of all treated rabbits. Severity was dose related. At the high dose ulceration was observed in five animals and necrosis was present in one. Four of five high dose and 2 of 5 mid dose males exhibited suppression of sperm formation. One control animal had severely reduced spermatogenesis. The spermatogenic findings between the control and high dose differ in that the high dose animals exhibit aspermia.</p>
<u>Conclusions</u>	<p>Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study. Significant toxicity was observed at all concentrations of the test material tested</p>
<u>Data Quality</u>	<p>Reliable with restriction (Klimisch Code). Restriction due to the fact that the individual testes findings are not presented for the re-evaluation. An explanation is not provided as to why the testes were reexamined nor as to why they were found to be normal at the time of the initial examination.</p>
<u>References</u>	<p>Unpublished confidential business information</p>
<u>Other</u>	<p>Updated: 7/21/00 (RTA-064)</p>

Robust Summary 5-Repeated Tox-2

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 407
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1993
Species	Rat
Strain	Sprague-Dawley CD, 57 (males)- 64 (females) days old at receipt
Route of administration	Oral gavage (syringe and dosing tube)
Duration of test	28 days of treatment
Doses/concentration levels (dose volume)	0, 10, 50, 125, 250 and 500 mg/kg/day (5ml/kg)
Vehicle	Corn oil (5 ml/kg)
Sex	Males and females
Exposure period	28-day treatment duration
Frequency of treatment	7 days/week
Number of animals/sex/group	5 rats/sex/group
Post exposure observation period	None
Statistical methods	Body weight, food consumption, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included a one-way analysis of variance followed by Dunnett's test.
Dose rangefinding study	Yes (Five day repeated dose oral toxicity study)
Remarks field for test conditions	<p>Single oral doses were administered for 28 consecutive days using a gavage needle. Clinical observations were performed daily prior to dosing, at the time of dosing and approximately 1 hour following dosing. Viability checks were performed twice daily. Body weights were recorded every other day and prior to necropsy. Individual food consumption was measured weekly. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically. These included the adrenals, esophagus, stomach, intestine, gonads, accessory sex organs and gross lesions from the control and two highest dose levels.</p> <p>Deviations from the OECD 407 test guidelines include:</p> <ul style="list-style-type: none"> • A functional observational battery for neurotoxicity was not performed since this test was not part of the OECD 407 guideline at the time the study was performed. • Hematology and clinical chemistry parameters were not evaluated. • Limited microscopic pathology was performed.

<u>Results</u>	
Remarks	<p>Three males and one female at 500 mg/kg/day died between study days 6 and 16. These deaths were considered treatment related. One female at 125 mg/kg/day died on day 7. The lack of any mortality at the higher dose level of 250 mg/kg suggests that this death was not treatment related. All other animals survived the duration of the study. Test article related clinical signs included changes in fecal consistency and coloration, staining of various body surfaces, rales, salivation and aggressive behavior in the 125, 250 and 500 mg/kg/day males and females. Rales and salivation were also observed in the 50 mg/kg/day males. Body weight gain was inhibited in the 250 mg/kg/day males and the 500 mg/kg/day males and females during the first two days of dosing. Reduced body weight gains were observed in the high dose males through study day 12. Food consumption was slightly reduced in the 500 mg/kg/day males and females during the first week of dosing. At necropsy a thickened mucosa of the nonglandular stomach was observed in one 500 mg/kg/day male and in one and two females at 250 and 500 mg/kg/day, respectively. Upon microscopic examination one 250 mg/kg/day male and all high dose females had submucosal edema on the glandular and/or non-glandular portions of the stomach. Three high dose females also had suppurative inflammation, primarily in the non-glandular portion of the stomach. These findings suggest a response to a gastric irritant. Mean absolute and relative adrenal weights in the 250 and 500 mg/kg/day males and females were increased. No histopathological lesions were associated with these increases.</p>
<u>Conclusions</u>	The Study Director concluded that the NOAEL for systemic toxicity was 10 mg/kg/day.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/21/00 (RTA-062)

Robust Summary 5-Repeated Tox-3

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu, isooctyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 410
Test Type	3 week dermal toxicity study in rabbits
GLP (Y/N)	N
Year (Study Performed)	1983
Species	Rabbit
Strain	New Zealand White
Route of administration	Dermal, 5 days/week, to the clipped, unabraded, dorsal surface.
Duration of test	15 days of treatment followed by a six week recovery period
Doses/concentration levels	Untreated control, sham control, vehicle control, 3 , 5 , 25 and 100%
Vehicle control	Base Oil Vehicle
Dose volume	2 mL/kg/day
Sex	Males
Frequency of treatment	Once/day, 5 days/week for a total of 15 doses.
Vehicle control and treatment groups	18 rabbits/group. Eight of the initial 18 animals/group served as recovery animals. The test material was administered undiluted to the treated animal in the high dose group. The animals in the lower dose group received the test material diluted in the vehicle. Doses were administered based on individual animal body weights.
Post exposure observation period	6 weeks
Statistical methods	Body weight, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared pretreatment vs post treatment. Tests included ANOVA and Tukey's B Test.
Remarks field for test conditions	The test material was applied to the clipped, unabraded dorsal surface of the rabbits 5 days/week for 3 weeks. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the Guideline.) Clinical observations were made 3 hours post dosing. Recovery animals were examined daily for the first week of recovery and once weekly thereafter. Dermal responses were evaluated weekly during treatment and immediately prior to sacrifice (Draize). Body weight was recorded weekly during treatment and recovery. (OECD Guideline 410 suggests the recording of food consumption. This parameter was not recorded during this study. This is considered a minor deviation from the guideline.) Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment and recovery. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically in the control and low dose

	animals.
<u>Results</u>	
Remarks	<p>All animals treated at the 100% dose level, 15 of 18 in the 25% and 1 of 18 in the vehicle and 5% dose groups died or were sacrificed moribund. No deaths occurred in the 3%, sham or untreated control groups. During recovery three animals in the vehicle control and one at 5% were sacrificed moribund. The three surviving animals at the 25% dose level were sacrificed at termination of the treatment period.</p> <p>Erythema and edema were observed in all treated rabbits within 24 hours of the first application. Severity was proportional to test material concentration and duration of exposure. Severe erythema and /or eschar formation were observed at later scoring periods. Similar results were noted in the vehicle control animals. Other findings included hyperirritability, diarrhea, decreased motor activity, ataxia, loss of righting reflex, ocular discharge, redness in the genital area and rippling of skin.</p> <p>Severe body weight losses were seen at the 100% and 25% dose levels. Significant decreases in body weight of the vehicle control, 3% and 5% groups relative to untreated and/or sham control were noted throughout the study. Differences between vehicle control and the 3 and 5% dose groups were not statistically significant. Recovery from body weight losses began following termination of treatment with the vehicle or the test material.</p> <p>Significant reductions in several hematology parameters (RBC, HGB, HCT, MCH, MCHC) were noted in the vehicle and test material treated groups at the termination of treatment. Increases in the percentages of mature heterophiles and corresponding decreases in lymphocytes were detected.</p> <p>Mean absolute weights of testes, prostate and epididymis were significantly lower in the vehicle and test material treated groups than in the sham or untreated control groups. Differences in weight between vehicle and test material treated animals were not significant. Vehicle and test material related effects noted on the skin, hematology parameters, and weight of reproductive organs observed during treatment were no longer significant at the end of the 6-week recovery period.</p> <p>Gross necropsy findings observed in the vehicle and treated animals following the treatment period included discoloration of the dosing site, scaling, scabbing, hair loss, skin thickening. Treated animals also exhibited enlargement of the prefemoral lymph node, discoloration of the lungs and liver red foci in the gastric mucosa and trichobezoars in the stomach. One animal in each of the 5, 25% and vehicle control groups had small soft testes. Necropsy observations following recovery included less frequent and less severe skin lesions, enlargement of the prefemoral and respiratory lymph nodes and heart and lung and liver discoloration. In the vehicle control one rabbit had small prostate, seminal vesicles and coagulation glands. Two rabbits had mesenteric fat necrosis and three rabbits had small testes. In general vehicle treated animals had a higher incidence of lesions following recovery than did the treated animals.</p>

	<p>The skin of the vehicle and treated animals had acanthosis and acute and severe inflammation with pus formation at the treatment site. In the test material treated animals the acanthosis was followed by escharotic chemical dermatitis which involved the deeper layers of the skin and resulted in blood and fluid loss. Following the six-week recovery period the lesions of the epidermis had abated. A low incidence of orchitis and maturation arrest at the primary spermatocyte level was observed in the testes of vehicle and test material treated animals following the treatment and recovery periods. Atypical pneumonia and lymphadenopathy were also seen in these rabbits. These changes were also noted in the other study groups and therefore were not considered related to treatment.</p>
<u>Conclusions</u>	<p>Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study. Significant toxicity was observed at all concentrations of the test material tested. Effects noted at 3 and 5% of the test material were similar to those observed with the vehicle. Suggesting that toxic effects observed at these dose levels were principally due to the vehicle.</p>
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/21/00 (RTA-063)

Robust Summary 5-Repeated Tox-4

<u>Test Substance</u>	
CAS #	113706-15-3
Chemical Name	Phosphorodithioic acid, secondary butyl and isooctyl) mixed esters, Zn Salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rabbits
GLP (Y/N)	Y
Year (Study Performed)	1980
Species	Rabbit
Strain	New Zealand White (SPF)
Mean Weight at Initiation of Dosing	Males 2.1 kg. Females 2.3 kg.
Route of administration	Dermal, 5 days/week, to the clipped, unabraded, dorsal surface.
Duration of test	20 days of treatment
Doses/concentration levels	0, 5 and 25% (w/v) (OECD Guideline 410 suggests three treated groups and a control be included in this study design. The lowest dose level should be free of toxic effects. These suggestions were not met in this study. This was a two-treated group study. Effects were seen at the lowest dose level.)
Vehicle control	Yes (Primol 185)
Dose volume	2 mL/kg/day
Analytical confirmation of dose concentration	Samples were sent to the Sponsor for analysis.
Sex	Males and females
Frequency of treatment	Once/day, 5 days/week for a total of 20 doses.
Vehicle control and treatment groups	Ten male and ten female rabbits in the vehicle control group and in both treated groups. An untreated control group was not included in the study.
Post exposure observation period	None
Statistical methods	Body weight, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to vehicle control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend.
Remarks field for test conditions	The test material was applied to the clipped, unabraded dorsal surface of the rabbits for, 5 days/week for 20 days. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the Guideline.) The backs of all animals were gently wiped with paper towels approximately 6 hours after exposure to remove excessive test substance, if necessary. Mortality and gross signs of toxicological and pharmacological effects were evaluated twice daily. Clinical examinations were made weekly. Dermal responses were evaluated daily (7 days/week). Body weight was recorded weekly during treatment. Hematology and clinical chemistry parameters were evaluated pretest and at termination of

	<p>treatment. Macroscopic examinations were performed on all animals. Select organs were weighed. Select tissues were examined microscopically for all animals in the vehicle control and high dose group. The testes were examined for all low dose males.</p>
<u>Results</u>	
Remarks	<p>Three high dose males and one high dose female were found dead or sacrificed moribund prior to study termination. These animals were found dead or were sacrificed on test days 8, 14, 17 and 18. One low dose males was found dead on test day 21. The cause of these deaths was not determined. All of the remaining vehicle control and treated animals survived the four-week duration of the study.</p> <p>Emaciation was a common finding in the 5% and 25% treated males and females. The incidence and severity of this finding were dose related. The high dose females were most affected. Dermal thickening was observed in all of the high dose animals during the last two weeks of study. Lacrimation was frequently observed in the high dose males and in the low and high dose females during the last two weeks of study. Lacrimation was observed in 2 vehicle control males and in 6 vehicle control females during the last week of the study. Ano-genital staining was observed in the high dose males and females.</p> <p>Low and high dose males and females generally exhibited marked, dose related increases in the incidence and severity of erythema, edema, atonia desquamation, fissuring, eschar formation and exfoliation. Many of these observations were moderate to extreme in severity, particularly those in the high dose group. Dermal irritation was observed in the vehicle control group. Severity and incidence were lower in the vehicle controls than in the test material treated animals.</p> <p>The mean body weights of the high dose males and females were reduced compared to vehicle control throughout the study. The high dose animals exhibited a loss in mean body weight over the treatment period. At study termination differences from vehicle control were –17% in the males and –20% in the females. The mean body weights of the low dose males and females were slightly reduced compared to vehicle control during the treatment period. Differences from vehicle control at termination were –2.9% in the males and –2% in the females.</p> <p>Mean hemoglobin, hematocrit and erythrocyte counts were statistically significantly reduced in the high dose males and females compared to vehicle control at study termination. In addition the mean platelet count of the high dose males was significantly elevated compared to vehicle control at study termination. The low and high dose males and females exhibited slight (low dose) to statistically significant (high dose) increases in mean cholesterol levels at termination. In addition statistically significant decreases were observed in the mean albumin levels of the high dose males and females. Total protein and albumin/globulin ratios were normal. The mean plasma, erythrocyte and brain cholinesterase values of the low and high dose females and high dose males were reduced compared to the vehicle control. Differences from vehicle control were for the most part statistically significant and ranged from –12% to –39%.</p>

	<p>The differences cited from vehicle control in hematology, clinical chemistry and cholinesterase parameters were considered treatment related.</p> <p>The mean and relative (to body weight) testes and epididymidal weights were markedly lower than vehicle control in the high dose group. The mean absolute testes weight was reduced 47% and the mean absolute epididymidal weight was reduced 43% compared to vehicle control. Testes and epididymidal weights in the low dose group were unremarkable. The mean absolute and relative (to body weight) adrenal weights of the high dose males and females were slightly to statistically significantly elevated compared to vehicle control. The mean absolute adrenal weight was increased 23% in the males and 46% in the females. The mean relative adrenal weights were increased 48% in the males and 80% in the females compared to vehicle control. Absolute and relative adrenal weights in the low dose males and females were considered comparable to vehicle control. Slight to statistically significant, dose related increases in mean absolute and relative kidney weights were evident in the low and high dose males and females.</p> <p>Macroscopic examinations conducted at study termination confirmed the in life dermal observations of the treated animals. Macroscopic dermal changes included atonia, alopecia, exfoliation, fissuring and eschar formation. In addition the testes of the high dose animals were observed to be markedly smaller than those of the vehicle control males.</p> <p>Compound related microscopic changes observed in the low and high dose males and females consisted of slight to moderately severe hyperkeratosis, parakeratosis and epithelial hyperplasia. In some rabbits, these changes were accompanied by an increase in the amount of collagen present in the dermis and/or focal to multifocal areas of suppurative dermatitis. The degree of severity of these lesions was greater at the high dose level. There was no demonstrable difference between the skin changes of the male and female rabbits.</p> <p>Microscopic examination of the testes from both dose groups determined the presence of morphologic abnormalities in the seminiferous tubules of the testes of the high dose animals only that were characterized by aspermatogenesis, diffuse tubular hypoplasia and a reduced mitotic activity. These findings were considered treatment related. No treatment related microscopic findings were observed in the adrenal glands of the high dose males or females.</p> <p>A number of the vehicle control and treated animals exhibited congestion and edema of the lungs. Subchronic inflammatory changes consisting of multifocal pneumonitis were present in some vehicle control and treated animals. None of the pulmonary findings were attributed to the topical administration of the test material.</p>
<u>Conclusions</u>	Based on the findings observed during this study, at the low dose level, this reviewer has concluded that an NOAEL was not established for this study.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 12/30/01

Robust Summary 5-Repeated Tox-5

<u>Test Substance</u>	
CAS #	28629-66-5
Chemical Name	isooctyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rabbits
GLP (Y/N)	Y
Year (Study Performed)	1980
Species	Rabbit
Strain	New Zealand White (SPF)
Route of administration	Dermal, 5 days/week, to the clipped, unabraded, dorsal surface.
Duration of test	20 days of treatment
Doses/concentration levels	0, 5 and 25% (w/v) (OECD Guideline 410 suggests three treated groups and a control be included in this study design. The lowest dose level should be free of toxic effects. These suggestions were not met in this study. This was a two-treated group study. Effects were seen at the lowest dose level.)
Vehicle control	Yes (Primol 185)
Dose volume	2 mL/kg/day
Analytical confirmation of dose concentration	Yes
Sex	Males and females
Frequency of treatment	Once/day, 5 days/week for a total of 20 doses.
Vehicle control and treatment groups	Ten male and ten female rabbits in the vehicle control group and in both treated groups. An untreated control group was not included in the study.
Post exposure observation period	None
Statistical methods	Body weight, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test and regression analysis for linear response, non-parametric Kruskal-Wallis and Dunn's Summed Rank Test, Jonckheere's test for monotonic trend.
Remarks field for test conditions	The test material was applied to the clipped, unabraded dorsal surface of the rabbits for, 5 days/week for 20 days. Elizabethan collars were used to prevent ingestion. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the Guideline.) The backs of all animals were gently wiped with paper towels approximately 6 hours after exposure to remove excessive test substance, if necessary. Mortality and gross signs of toxicological and pharmacological effects were evaluated twice daily. Clinical examinations were made weekly. Dermal responses were evaluated daily (7 days/week). Body weight was recorded weekly during treatment. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. Select organs were weighed. Select tissues were examined microscopically for all

	animals in the vehicle control and high dose group. The testes and gross changes and tissue masses were evaluated for all low dose animals.
<u>Results</u>	
Remarks	<p>One high dose male and three high dose females died prior to study termination. These animals were found dead on test days 17, 23, 29 and 30. The cause of these deaths was not determined. All of the remaining vehicle control and treated animals survived the four-week duration of the study.</p> <p>Emaciation was a common finding in the 5% and 25% treated males and females. The incidence and severity of this finding were dose related. The high dose females were most affected. Dermal thickening was observed in most of the treated animals during the last two weeks of study. Ano-genital staining, nasal discharge and lacrimation were frequently observed in the treated males and females at both dose levels. Lacrimation was observed in 2 vehicle control males and in 6 vehicle control females during the last week of the study.</p> <p>Low and high dose males and females generally exhibited marked, dose related increases in the incidence and severity of erythema, edema, atonia desquamation, fissuring, eschar formation and exfoliation. Many of these observations were moderate to extreme in severity, particularly those in the high dose group. Dermal irritation was observed in the vehicle control group. Severity and incidence were lower in the vehicle controls than in the test material treated animals.</p> <p>The mean body weights of the low and high dose males and females were reduced compared to vehicle control throughout the study. Differences from vehicle control in the low and high dose males ranged from 3 to 17%, and in the low and high dose females ranged from 10 to 23%. These differences from vehicle control were considered treatment related.</p> <p>Mean hemoglobin, hematocrit and erythrocyte counts were slightly to statistically significantly reduced compared to vehicle control at study termination. Slight to statistically significant differences from vehicle control were observed in several clinical chemistry parameters of the test material treated animals. These included: decreased alkaline phosphatase (25% females), decreased albumin (25% males/females), increased blood urea nitrogen (25% males/females), increased cholesterol (5 and 25% males/females) and increased total and direct bilirubin (5 and 25% females). In addition mean brain cholinesterase levels were slightly reduced (-14 to -17%) in the 5 and 25% males and females. These differences from vehicle control in hematology and clinical chemistry parameters were considered treatment related.</p> <p>The mean and relative (to body weight) testes and epididymides weights were markedly lower than control in the low and high dose groups. Differences from control were dose related and were considered treatment related.</p> <p>Macroscopic and microscopic examinations conducted at study termination confirmed the in life dermal observations of the treated animals. Microscopic dermal changes consisted of slight to moderately severe hyperkeratosis,</p>

	<p>parakeratosis and epithelial hyperplasia. In some rabbits, these changes were accompanied by an increase in the amount of collagen present in the dermis and/or focal to multifocal areas of suppurative dermatitis. The degree of severity of these lesions was greater at the high dose level. There was no demonstrable difference between the skin changes of the male and female rabbits. At necropsy the testes of the 5 and 25% dose group animals were markedly smaller than those of the vehicle controls. Microscopic examination of the testes from both dose groups determined the presence of morphologic abnormalities in the seminiferous tubules of the testes that were characterized by aspermatogenesis, diffuse tubular hypoplasia and a reduced mitotic activity. These findings were considered treatment related.</p>
<u>Conclusions</u>	Based on the findings observed during this study, at the low dose level, this reviewer has concluded that an NOAEL was not established for this study.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 9/13/01

Robust Summary 5-Repeated Tox-6

<u>Test Substance</u>	
CAS #	CAS# 2215-35-2
Chemical Name	2-pentanol, 4-methyl-, hydrogen phosphorodithioate, Zn salt
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	43 CFR 163.82-2
Test Type	3 week dermal toxicity study in rabbits
GLP (Y/N)	N
Year (Study Performed)	1979
Species	Rabbit
Strain	Albino
Route of administration	Dermal, 5 days/week, to the clipped, dorsal surface.
Duration of test	Three weeks, 15 days of treatment
Doses/concentration levels	0, 0.8 and 1.6 ml/kg
Vehicle control	None
Sex	Males/Females
Frequency of treatment	Once/day, 5 days/week for a total of 15 doses.
Control and treatment groups	3/sex/group unabraded 3/sex/group abraded (Due to an apparent incorrect sexing 2 abraded control males and 4 abraded control females were placed on study.)
Post exposure observation period	None
Statistical methods	Analysis of variance, Newman-Keuls analysis
Remarks field for test conditions	The test material was applied to the clipped, unabraded or abraded dorsal surface of the rabbits 5 days/week for 3 weeks. Abraded animals were abraded once per week. The treated skin was covered with gauze patches secured with hypoallergenic tape, and covered with an impervious wrap that was held in place with an elastic bandage. Treatment sites were occluded for 6 hours/day, 5 days/week. Control animals were handled in an identical fashion as the treated animals, however, they were untreated. Clinical observations were made daily. Dermal responses were evaluated daily, on treatment days, immediately prior to the next application of test material (Draize). Body weights and food consumption were determined every 3 to 4 days. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. Select tissues were weighed. A range of tissues was examined microscopically for all animals.
<u>Results</u>	
Remarks	A total of three males and three females were found dead or were sacrificed moribund during the study. Two high dose males (Group II, unabraded) were sacrificed moribund on test days 16 and 19 respectfully. The cause of death of these animals was not determined. One high dose female (Group II, unabraded) died during terminal bleeding. The cause of death was attributed to pneumonia. One low dose female (Group I, unabraded) died on test day 21. The pathologist attributed this animal's death to infection and pneumonia. One Control female (Group III, abraded) died on test day 22 following bleeding. Death was

attributed to hemorrhage following intracardiac bleeding. One Control male (Group III, abraded) was found dead on test day 15. This animal's death was probably due to the presence of severe pneumonitis.

Dermal irritation (slight to moderate erythema and edema) was present on test day 1 in both abraded and unabraded low and high dose male and female animals. By test days 3 - 4 severe erythema and severe edema were present in the abraded and unabraded males and females at both dose levels. Skin cracking was observed in all treated animals by the end of the first week of study. The severity of findings was essentially the same in the low and high dose animals. Differences in findings by dose level were not apparent.

All of the abraded and unabraded high dose males and females exhibited a body weight loss over the 21-day study period. The low dose unabraded female that died during the study exhibited a progressive weight loss throughout the study up until the time it died on test day 21. Two of three unabraded low dose males and one of the two surviving low dose unabraded females lost weight during the treatment period. All of the abraded low dose males and 2 of 3 abraded low dose females gained weight during the study. The remaining abraded low dose female lost weight during the last ten days of study. In general the mean food consumption values of the surviving low and high dose animals were lower than control, suggesting a dose related effect of test material administration.

The mean total white blood cell count was elevated in the males in the low (unabraded only) and high (abraded and unabraded) dose groups. The increases observed in the high dose males were attributed to treatment. Observed alterations in clinical chemistry parameters included: triglyceride- increased in the high dose, abraded and unabraded, males and females; uric acid- increased in the high dose, abraded and unabraded, females; SGOT- increased in the high dose abraded females, only and LDH- increased in the high dose, abraded and unabraded, females. These increases, in the high dose group only, suggest a relationship to treatment with the test material. The mean GGT level of the high dose abraded females was also elevated compared to control. This increased value was attributed to an increase in one of the three abraded high dose animals only. This increase was not considered treatment related.

The mean testes weights, testes/body weight ratios and testes to brain weight ratios of the high dose, abraded and unabraded, males were lower than control. These differences from control were considered treatment related. The liver and heart weights and the liver and heart to brain weight ratios of the high dose males (abraded and unabraded) were reduced compared to controls. The differences from control in heart and liver weights were not associated with any microscopic findings in these organs. For this reason, these differences from control (heart and liver) were not attributed to treatment. The mean ovary weights, ovary/body weight ratios and ovary to brain weight ratios of the high dose, abraded and unabraded, females were lower than control. The differences from control in absolute and relative ovary weights were not associated with any microscopic findings. For this reason, these differences from control were not attributed to treatment.

	<p>Skin necrosis with epithelial hyperplasia upon re-epithelialization, subacute interstitial pneumonia and depletion of lymphoid organs due to stress and weight loss were observed microscopically in both the low and high dose groups. The findings were more severe in the high dose. No significant microscopic pathology was observed in the heart, liver, gastrointestinal tract, kidneys, urinary bladder, adrenals, thyroid or brain.</p> <p>Of the six male animals in the high dose, the testes of the three rabbits with unabraded skin showed decreased spermatogenesis, the testes of one rabbit with abraded skin showed increased multinucleate forms. The testes of the three male high dose animals with abraded skin exhibited no pathologic changes. No inflammatory changes were seen in the testes of the animals which showed reduced or altered spermatogenesis. Similar changes were not seen in either the control or low dose group except for two low dose males that had slightly reduced spermatogenic activity. The decreased spermatogenic activity observed in the high dose group was attributed to exposure to the test material.</p>
<u>Conclusions</u>	Based on the findings observed during this study this reviewer has concluded that an NOAEL was not established for this study. Significant toxicity was observed at both concentrations of the test material tested
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the fact that this study predates the EPA GLPs and that, physical observation data, other than irritation findings, were not reported. In addition many of the animals had evidence of respiratory disease that was common in rabbit studies at the time that this study was conducted.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 12/10/01

2.3 Genetic Toxicity

Robust Summary 5-GenTox-1

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	In vitro point mutation assay using BALB/3T3 mouse embryo cells without metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1981
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 30, 10 and 3 ug/mL were evaluated without metabolic activation.
Metabolic Activation	None
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in aqueous cell culture medium. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG): 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the mutation assay. Plates were incubated at 36° C in a humidified atmosphere of 5% CO ₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After the 4-6 day expression period necessary for fixation of the genotypic lesion which evolves as the mutant phenotype, the number of cells in a representative mutation assay plate for each test condition was determined in order to establish the number of cells at risk to the selective agent (ouabain). Cells in the remaining mutation assay plates were then treated with complete medium containing 1mM ouabain. Cells were incubated at 36° C with scheduled medium/ouabain changes for 4-6 weeks. Cells were then fixed, stained and scored for the development of ouabain-resistant colonies.

	<p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The number of colonies forming/number of cells seeded/treatment condition were determined for the positive and negative controls and the treated groups. The CAR (number of cells at risk to ouabain) for each treatment group was calculated from the cell counts of representative mutation assay plates for each group. The mutation results were expressed in terms of mutation frequencies (the number of ouabain resistant mutant colonies/number of CAR/condition).</p> <p>For a valid test the cloning efficiency of the solvent control must be greater than or equal to 15%. The relative survival of cells exposed to the test article must be equal to or greater than 40% for at least one dose level. The number of spontaneous ouabain-resistant mutants in the negative control must not exceed 4/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of ouabain-resistant mutants relative to the negative control.</p>
<u>Results</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the absence of metabolic activation.
Remarks	Relative to solvent control cell survival was 85%, 67% and 0.3% at 3, 10 and 30 ug/ml respectively. The positive control reduced the colony forming efficiency of the 3T3 cells by 97%. Three spontaneous ouabain-resistant colonies were observed in the vehicle control. The test material induced 132 ouabain-resistant colonies at 10 ug/ml and 16 at 3 ug/ml. Mutation frequencies in these groups and in the positive control were statistically significant compared to the vehicle control.
<u>Conclusions</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the absence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/7/00 (RTA-067)

Robust Summary 5-GenTox-2

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	In vitro point mutation assay using BALB/3T3 mouse embryo cells with metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1981
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 3, 1 and 0.3 ug/mL were evaluated with metabolic activation.
Metabolic Activation	Yes
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in aqueous cell culture medium. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	Benzo(a)pyrene, 12.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31 cells were seeded for the evaluation of cytotoxicity and mutagenicity at 250 cells/culture in triplicate/condition. Cells were treated in suspension in a reaction mixture containing the cofactor pool (NADPH), S-9 and test article or control compounds and were incubated at 36° C in a humidified atmosphere of 5% CO₂ in air for 2 hours. After the exposure period, the target cells were washed with HBSS and resuspended in complete medium.</p> <p>For the cytotoxicity assay, cells were seeded in complete medium at 250 cells/plate in triplicate/condition for the determination of the relative cytotoxic effects of each treatment. Cells were incubated at 36° C with scheduled medium changes for approximately 7 to 10 days. The cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation in order to determine the relative plating efficiency per condition.</p> <p>For the mutation assay, the remaining cells from each test condition were cultured further in complete medium at 36° C for the 4-6 day expression period necessary for fixation of the genotypic lesion, which evolves as the mutant phenotype. The cells were then collected and an aliquot from each condition was seeded at 250 cells/plate in triplicate/condition and incubated at 36° C with scheduled medium changes. After approximately 7 to 10 days, the plates were</p>

	<p>fixed with methanol, stained with 10% Giemsa, and scored for colony formation in order to determine the relative plating efficiency per condition and in order to establish the number of cells at risk to the selective agent (ouabain). Cells in the remaining mutation assay plates were then treated with complete medium containing 1mM ouabain.</p> <p>Induction of mutation was determined by seeding approximately 2×10^5 cells/dish, in complete medium, from those remaining cells collected post expression, into 12-15 dishes/condition. Plates were incubated for 4 hours. Ouabain (5mM) was added to each culture to a final concentration of 1 mM in complete medium. Cells were incubated with ouabain/medium changes for 4-6 weeks. Cells were then fixed, stained and scored for the development of ouabain-resistant colonies.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The number of colonies forming/number of cells seeded/treatment condition were determined for the positive and negative controls and the treated groups. The CAR (number of cells at risk to ouabain) for each treatment group was calculated from the cell counts of representative mutation assay plates for each group. The mutation results were expressed in terms of mutation frequencies (the number of ouabain resistant mutant colonies/number of CAR/condition).</p> <p>For a valid test the cloning efficiency of the solvent control must be greater than or equal to 15%. The relative survival of cells exposed to the test article must be equal to or greater than 40% for at least one dose level. The number of spontaneous ouabain-resistant mutants in the negative control must not exceed 4/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of ouabain-resistant mutants relative to the negative control.</p>
<u>Results</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the presence of metabolic activation.
Remarks	Relative to solvent control cell survival was 94%, 81% and 80% at 0.3, 1.0 and 3.0 ug/ml respectively. The positive control reduced the colony forming efficiency of the 3T3 cells by 26%. Four spontaneous ouabain-resistant colonies were observed in the vehicle control. The test material induced 11 ouabain-resistant colonies at 3 ug/ml, 8 at 1 ug/ml and 21 at 0.3 ug/ml. Mutation frequencies in these groups and in the positive control were statistically significant when compared to the vehicle control.
<u>Conclusions</u>	The test substance exhibited positive mutagenic activity in 3T3 cells in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 2/23/01 (RTA-075)

Robust Summary 5-GenTox-3

<u>Test Substance</u>	
CAS #	CAS# 26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Mutation Research 31: 9-15 (1975)
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1980
Species	Mouse
Strain	Swiss Albino CrI:CD-1
Route of administration	Intraperitoneal
Duration of test	Two doses 24 hours apart followed by a 6-hour evaluation period.
Doses/concentration levels	0, 10 and 20 mg/kg
Dose volume	20 ml/kg
Sex	Males and females
Frequency of treatment	Twice (at initiation and at 24 hours)
Control and treatment groups	0.25% Methylcellulose and Tween 80 vehicle control: 4/sex; triethylenemelamine positive control: 0.5 mg/kg, 4/sex; 10 and 20 mg/kg: 4/sex
Statistical methods	Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls using a Student's t test.
Dose rangefinding study	10, 30, 100, 300 and 6000 mg/kg Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. All animals from each treatment group and vehicle control group were sacrificed for bone marrow sampling 6 hours after the last treatment. The frequency of micronucleated cells was expressed as percent micronucleated cells versus total polychromatic erythrocytes. (This study design deviates significantly from OECD Guideline 474. Differences include the number of administrations of test material (2x vs 1x), number of animals/sex (4 vs 5), number of dose levels evaluated (2 vs 3), and number of evaluation time points (1 vs 3).)
<u>Results</u>	
Remarks	<p>During the dose rangefinding study significant mortality was observed at all levels above 30 mg/kg. The 10 and 30 mg/kg dose levels exhibited hypoactivity and writhing after dose administration. Based on these data dose levels of 10 and 20 mg/kg were selected for the main study.</p> <p>During the main study test material treated animals exhibited hypoactivity and piloerection post dosing. No deaths occurred. No statistically significant increases in micronucleated polychromatic</p>

	erythrocytes over the levels observed in the vehicle controls were observed in either sex. The variability in response observed in the treated animals was similar to that observed in the vehicle control. The positive control exhibited a statistically significant increase in micronuclei as expected.
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction based on the number and type of deviations in the study design from OECD Guideline 474.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-060)

Robust Summary 5-GenTox-4

<u>Test Substance</u>				
CAS #	CAS# 26566-95-0			
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts			
Remarks	Test material purity not provided.			
<u>Method</u>				
Method/Guideline followed	OECD Guideline 471			
Test Type	Bacterial Reverse Mutation Assay			
GLP (Y/N)	N			
Year (Study Performed)	1981			
Test System	<i>Salmonella typhimurium</i>			
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537, TA1538			
Exposure Method	Plate incorporation			
Test Substance	0.1, 0.03, 0.01, 0.003 and 0.001 ul/plate without activation			
Doses/concentration levels	0.3, 0.1, 0.03, 0.01 and 0.003 ul/plate with activation			
Metabolic Activation	With and without (S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)			
Vehicle	Ethanol			
Tester strain, activation status, Positive Controls and concentration level	TA98	+S9	2-aminoanthracene	5 ug/plate
	TA98	-S9	2-nitrofluorene	5 ug/plate
	TA100	+S9	2-aminoanthracene	5 ug/plate
	TA100	-S9	sodium azide	30 ug/plate
	TA1535	+S9	2-aminoanthracene	5 ug/plate
	TA1535	-S9	sodium azide	30 ug/plate
	TA1537	+S9	2-aminoanthracene	5 ug/plate
	TA1537	-S9	9-aminoacridine	10 ug/plate
	TA1538	+S9	2-aminoanthracene	5 ug/plate
	TA1538	-S9	2-nitrofluorene	5 ug/plate
Vehicle Control	Acetone			
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.			
Dose Rangefinding Study	No			
S9 Optimization Study	No			
Remarks field for test conditions	<p>This study was conducted prior to the development of OECD Guideline No. 471. This study deviates from the guideline in that Tester Strain TA 1538 is not called for in the guideline but it was included. In addition E. coli WP2 urvA Tester Strain called for in the guideline was not include.</p> <p>There were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 100 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if</p>			

	needed) and 2.0 ml of top agar. This was overlaid onto the surface of minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>All data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation.</p> <p>The positive control for each respective test strain exhibited at least a 5-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.</p>
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code). Restriction due to the lack of any information regarding the selection of dose levels used during the study. In addition no information is presented regarding cytotoxicity or the presence of test material precipitate in the cultures.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/16/00 (RTA-056)

Robust Summary 5-GenTox-5

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino Crl:CD-1 (ICR)BR
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 6, 12 and 24 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; cyclophosphamide positive control: 60 mg/kg, 5/sex; 6 and 12 mg/kg: 15/sex; 24 mg/kg: 20/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes was evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	Study I: 550, 1787, 3024, 4261 and 5498 mg/kg Study II: 25, 37.5, 50, 75 and 100 mg/kg Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of ≥ 1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to effect the reliability of the study.)
<u>Results</u>	
Remarks	During the first dose rangefinding study significant mortality was observed at all levels. Hypoactivity and signs of moribundity were observed in most animals at all dose levels. During the second dose

	<p>range-finding study significant mortality was observed at all levels. At the low dose (25 mg/kg) 1 male and all 3 females survived. Hypoactivity, tremors and/or squinting eyes were noted in a number of the treated animals. Based on these data dose levels of 0, 6.0, 12 and 24 mg/kg were selected for the main study.</p> <p>During the main study one 72-hour low dose female died shortly after dosing. Upon necropsy this death was attributed to a dosing accident. This animal was replaced. Between 20 hours post dosing and study termination 7 male and 2 female high dose animals were found dead. Several of these animals exhibited hypoactivity, tremors and were prostrate prior to death. Slight hypoactivity was observed at 12 mg/kg and higher on the day of dosing.</p> <p>No statistically significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls were observed in either sex or at any harvest time point. All values for individual animals were within the expected range of micronucleated-PCE/1000 PCE expected for control animals. The variability in response observed in the treated animals was similar to that observed in the vehicle control. The positive control exhibited a statistically significant increase in micronuclei as expected. The test article did induce a statistically significant decrease in the PCE:NCE ratio of the high dose females at 48 hours.</p>
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-059)

Robust Summary 5-GenTox-6

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537; <i>Escherichia Coli</i> tester strain WP2uvrA
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	<u>Initial assay:</u> <i>Salmonella</i> + (S9): 25, 50, 100, 250, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 1.0, 5.0, 10.0, 25.0, 100, and 500 ug/plate WP2uvrA + (S9): 50, 100, 250, 500, 2,000, and 10,000 ug/plate WP2uvrA - (S9): 50, 100, 250, 500, 2,000 and 10,000 ug/plate <u>Confirmatory assay:</u> <i>Salmonella</i> + (S9): 50, 100, 250, 500, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 5.0, 10.0, 25.0, 50.0, 100 and 500 ug/plate WP2uvrA + (S9): 100, 250, 500, 2,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 50, 100, 250, 500, 2,000 and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2uvrA +S9 2-aminoanthracene 25.0 ug/plate WP2uvrA -S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Vehicle Control	Ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2uvrA and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a cytotoxic dose level of test article (66.7 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was

	evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. Plates that were not evaluated immediately were held at 5°C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>In the dose rangefinding study cytotoxicity was observed with tester strain TA100 at 333 ug/plate and above with metabolic activation and at 33.3 ug/plate and above without metabolic activation. With tester strain WP2uvrA cytotoxicity was observed at 667 ug/plate without activation and at 6,670 ug/plate with metabolic activation. Test article precipitate was observed on plates at 6,670 ug/plate and above with tester strain TA100 with and without activation. With WP2uvrA with metabolic activation precipitate was observed at 667 ug/plate and above. Without activation, with WP2uvrA, precipitate was observed at 3,330 ug/plate. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected.</p> <p>The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (333 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study.</p> <p>In the initial and confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on the results of the initial study the dilution factor between doses was reduced for the confirmatory study in the absence of metabolic activation. The doses outlined above (page 1, Test Substance Doses, Confirmatory Assay) were utilized.</p> <p>Cytotoxicity was observed at ≥ 50 ug/plate with the <i>Salmonella</i> tester strains with and without activation and at ≥ 250 ug/plate with WP2uvrA with and without activation. Test material participate was observed on plates at ≥ 500 ug/plate.</p> <p>The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control</p>

	response.
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/14/00 (RTA-054)

Robust Summary 5-GenTox-7

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino Crl:CD-1 (ICR)BR
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 12.5, 25 and 50 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; cyclophosphamide positive control: 60 mg/kg, 5/sex; 12.5 and 25 mg/kg: 15/sex; 50 mg/kg: 20/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes was evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	12.5, 25, 50, 75 and 100 mg/kg Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of ≥ 1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to effect the reliability of the study.)
<u>Results</u>	
Remarks	During the dose rangefinding study significant mortality was observed at the 75 and 100 mg/kg dose levels. Hypoactivity was observed in

	<p>some animals at all dose levels during the first hour post dosing and at 50 mg/kg and higher at 18 hours post dosing. Based on these data dose levels of 0, 12.5, 25 and 50 mg/kg were selected for the study.</p> <p>During the main study hypoactivity was observed at 25 mg/kg and higher. At 50 mg/kg three males and two females were found dead between 17 and 67 hours post dosing.</p> <p>A statistically significant increase in micronucleated PCEs was observed in the high dose total for males and females at the 72-hour harvest time point. This increase was not attributed to test material exposure and was due to the low number of micronucleated PCEs in the concurrent control group. There were no significant increases for the high dose males or females at the 72-hour harvest individually, no observable dose response, and the value was within the historical range for the testing facility. No other significant increases in micronucleated PCEs over vehicle control levels occurred at any other harvest time.</p> <p>The positive control exhibited a statistically significant increase in micronuclei as expected. The test article did induce a statistically significant decrease in the PCE:NCE ratio of the high dose males at 48 hours.</p>
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-058)

Robust Summary 5-GenTox-8

<u>Test Substance</u>	
CAS #	CAS# 68784-31-6
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(sec-Bu and 1,3-dimethylbutyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537; <i>Escherichia Coli</i> tester strain WP2uvrA
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	<u>Initial assay:</u> <i>Salmonella</i> + (S9): 50, 100, 250, 500, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 10, 25, 50, 100, 250 and 1,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 50, 100, 250, 500, 2,000 and 10,000 ug/plate <u>Confirmatory assay:</u> <i>Salmonella</i> + (S9): 50, 100, 250, 500, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 50, 100, 250, 500, 1,000 and 5,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitrofluorene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2uvrA +S9 2-aminoanthracene 25.0 ug/plate WP2uvrA -S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Vehicle Control	Ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Ranging Study	Conducted using tester strains TA100 and WP2uvrA and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a cytotoxic dose

	level of test article (333 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. Plates that were not evaluated immediately were held at 5°C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>In the dose rangefinding study cytotoxicity was observed with tester strain TA100 at 1,000 ug/plate and above with metabolic activation and at 333 ug/plate and above without metabolic activation. With tester strain WP2uvrA cytotoxicity was observed at 1,000 ug/plate without activation. Cytotoxicity was not observed with this tester strain with activation. Test article precipitate was observed on plates at 6,670 ug/plate and above with tester strain TA100 without activation. With TA100 with metabolic activation precipitate was observed on plates at 3,330 ug/plate and higher. With WP2uvrA with metabolic activation precipitate was observed at 667 ug/plate and above. Without activation, with WP2uvrA, precipitate was observed at 3,330 ug/plate. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected.</p> <p>The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (333 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study.</p> <p>In the initial and confirmatory mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on the results of the initial study the dilution factor between doses was reduced for the confirmatory study in the absence of metabolic activation. The doses outlined above (page 1, Test Substance Doses, Confirmatory Assay) were utilized.</p> <p>Cytotoxicity was observed at $\geq 1,000$ ug/plate with the <i>Salmonella</i> and WP2uvrA tester strains with and without activation. Test material participate was observed on plates at $\geq 1,000$ ug/plate. In one trial</p>

	<p>precipitate was observed in the presence of S9 mix at a dose as low as 250 ug/plate.</p> <p>The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.</p>
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/14/00 (RTA-053)

Robust Summary 5-GenTox-9

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu, isooctyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Assay
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y TK+/- mouse lymphoma cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 0.016, 0.012, 0.0089, 0.0067, 0.0050, 0.0038, 0.0028, 0.0021, 0.0016 and 0.0012 ul/mL were evaluated without metabolic activation. Concentrations of 0.032, 0.024, 0.018, 0.013, 0.010, 0.0075, 0.0056, 0.0042, 0.0032 and 0.0024 ul/mL were evaluated with metabolic activation.
Metabolic Activation	Aroclor induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 5 and 7.5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test.
Dose rangefinding study	Test substance (dose levels from 0.001 to 10 ug/mL) and vehicle control tested with and without activation. Cultures were exposed to the test substance and incubated for approximately four hours, then washed and cultured for two days. Cell culture density was determined 24 and 48 hours post exposure. Treated cell suspension growth at each dose level was compared to the negative solvent control.
Remarks field for test conditions	<p>Prior to study initiation the solubility of the test substance and of the positive control materials in the vehicle was confirmed.</p> <p>In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation. The test material was prepared so that the highest concentration was 100% toxic. The test material was added to cells with and without activation and incubated for four hours. Cells were then washed and placed in suspension cultures for two days with a cell population adjustment at 24 and 48 hours. The cells were then plated in a restrictive media containing trifluorothymidine (TFT) which allows TK^{-/-} cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability. Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies</p>

	in the corresponding viability plates. For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control with a dose response.
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation. The test substance was positive for mutagenic activity in the presence of metabolic activation.
Remarks	<p>The dose rangefinding study indicated a threshold level of complete toxicity at 0.05 ul/ml without activation and 0.1 ul/ml with activation. Based on these data the concentration ranges outlined above were selected for study in the main assay.</p> <p>In the main assay one culture cloned without activation exhibited a mutation frequency that was 4.8 times greater than the solvent control. This result was not considered significant since the total growth in this culture was 2%. None of the other cultures exhibited mutation frequencies that were significantly greater than control. The total growth of these cultures ranged from 12 to 109%. One metabolically activated culture (at the highest test material concentration tested) exhibited a mutation frequency that was significantly greater than the mutation frequency of the solvent controls. In addition, the cultures exhibited a dose response. Positive and vehicle control group responses were appropriate.</p>
<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation. The test substance was positive for mutagenic activity in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/18/00 (RTA-061)

Robust Summary 5-GenTox-10

<u>Test Substance</u>	
CAS #	CAS# 68988-46-5
Chemical Name	Phosphorodithioic acid mixed o,o-bis(iso-Bu, isooctyl, and pentyl) esters zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells without metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Concentrations of 10, 20 and 30 ug/mL were evaluated without metabolic activation.
Metabolic Activation	None
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in Eagle's minimal essential medium (EMEM). Solvent control plates were treated with acetone at a final concentration of 2 ul/ml complete EMEM.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG): 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes (clonal toxicity)
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31-1 cells were seeded for each treatment condition at 250 cells/ culture in 12-15 replicates for determination of phenotypic transformation and were incubated at 36° in a humidified atmosphere of 5% CO₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control must not exceed one focus/total replicate plates. The positive control must</p>

	induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.
<u>Results</u>	The test substance did not induce a statistically significant level of morphological transformation in BALB/3T3 cells.
Remarks	Relative to solvent control cell survival was 32%, 88% and 102% at 30, 20, and 10 ug/ml respectively. One spontaneous Type II but no Type III foci were observed in the solvent control. One Type II focus was observed at 30 ug/ml and one Type III focus was observed at 20 and 10 ug/ml each. The transformation frequencies were not statistically increased compared to the acetone control. The positive control induced 6 Type II and 14 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.
<u>Conclusions</u>	The test substance did not induce a statistically significant level of morphological transformation in BALB/3T3 cells.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 8/7/00 (RTA-064)

Robust Summary 5-GenTox-11

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino Crl:CD-1 (ICR)BR
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 7.13, 14.3 and 28.5 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; cyclophosphamide positive control: 60 mg/kg, 5/sex; 7.13 and 14.3 mg/kg: 15/sex; 28.3 mg/kg: 20/sex
Statistical methods	Animal to animal variability in spontaneous frequency of micronucleated polychromatic erythrocytes was evaluated in vehicle controls. Statistically significant differences were evaluated in the frequency of micronucleated polychromatic erythrocytes between treated groups and vehicle controls. NCE/PCE (normochromatic erythrocytes/polychromatic erythrocytes) ratios in treated and control groups were compared. Tests included Cochran-Armitage test for trend, a one-way analysis of variance and Dunnett's procedure.
Dose rangefinding study	Three rangefinding studies was conducted at the following dose levels: Study I: 554, 1802, 3049, 4296 and 5544 mg/kg Study II: 37.5, 62.5, 125 and 250 mg/kg Study III: 25, 12.5, 6.25, 3.13 and 1.0 mg/kg/day Mortality and physical observations were evaluated.
Remarks field for test conditions	All animals observed frequently for physiological or behavioral abnormalities on the day of dosing and periodically thereafter. Body weights taken on first day of the study prior to treatment. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls sampled at 24 hours only. NCE/PCE ratio and %PCE of total erythrocytes were calculated by counting a total of ≥ 1000 erythrocytes/animal. A total of 1000 PCE /animal were evaluated for the presence of micronuclei. (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to effect the reliability of the study.)

<u>Results</u>	
Remarks	<p>During the first two dose rangefinding studies significant mortality was observed at all dose levels. In the third rangefinding study 1 high dose male died. Hypoactivity was observed in some animals at all dose levels greater than 1 mg/kg. Based on these data dose levels of 0, 7.13, 14.3 and 28.5 mg/kg were selected for the study.</p> <p>During the main study hypoactivity was observed in all dose groups during the first two hours post dosing. At 72 hours post dosing one male in the 7.13 mg/kg dose group was found dead.</p> <p>No statistically significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls were observed in either sex or at any harvest time point. All values for individual animals were within the expected range of micronucleated-PCE/1000 PCE expected for control animals. The variability in response observed in the treated animals was similar to that observed in the vehicle control. The positive control exhibited a statistically significant increase in micronuclei as expected. The test article did not induce a statistically significant change in the PCE:NCE ratio.</p>
<u>Conclusions</u>	Under the conditions of this study the test material did not induce micronuclei in bone marrow erythrocytes of mice.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/17/00 (RTA-057)

Robust Summary 5-GenTox-12

<u>Test Substance</u>	
CAS #	CAS# 84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	<i>Salmonella typhimurium</i> and <i>Escherichia Coli</i>
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535, TA1537; <i>Escherichia Coli</i> tester strain WP2uvrA
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	Initial assay: <i>Salmonella</i> + (S9):25, 50, 100, 250, 1,000, and 5,000 ug/plate <i>Salmonella</i> - (S9): 10, 25, 50, 120, 600 and 3,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 25, 50, 100, 250, 1,250 and 6,500 ug/plate Confirmatory assay: <i>Salmonella</i> + (S9):100, 250, 500, 1,000, 2,500, and 5,000 ug/plate <i>Salmonella</i> - (S9): 50, 100, 250, 500, 1,000 and 3,000 ug/plate WP2uvrA + (S9): 100, 250, 500, 1,000, 5,000, and 10,000 ug/plate WP2uvrA - (S9): 100, 250, 500, 1,000, 2,000, and 6,500 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2uvrA +S9 2-aminoanthracene 25.0 ug/plate WP2uvrA -S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Vehicle Control	Ethanol
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2uvrA and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a cytotoxic dose level of test article (333 ug/plate) and four concentrations of S9 mix

	(5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37°C. Plates that were not evaluated immediately were held at 5°C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>In the dose rangefinding study cytotoxicity was observed with tester strain TA100 at 667 ug/plate and above with metabolic activation and at 100 ug/plate and above without metabolic activation. With tester strain WP2uvrA cytotoxicity was observed at 10,000 ug/plate with activation and at 667 ug/plate and above without activation. Test article precipitate was observed on plates at 6670 ug/plate and above with tester strains TA100 and WP2uvrA without activation. With TA100 with metabolic activation precipitate was observed on plates at 1000 ug/plate and higher. With WP2uvrA with metabolic activation precipitate was observed at 3330 ug/plate and above. Based on these results dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected.</p> <p>The S9 optimization study was performed using TA98 and TA100 with the highest non-cytotoxic dose of test article, (10,000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study.</p> <p>In the initial and confirmatory mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on the results of the initial study the dilution factor between doses was reduced for the confirmatory study. The doses outlined above (page 1, Test Substance Doses, Confirmatory Assay) were utilized.</p> <p>Cytotoxicity was observed at ≥ 500 ug/plate with the <i>Salmonella</i> and WP2uvrA tester strains with and without activation. Test material participate was observed on plates at $\geq 3,000$ ug/plate.</p> <p>The positive control for each respective test strain exhibited at least a</p>

	3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/14/00 (RTA-052)

Robust Summary 5-GenTox-13

<u>Test Substance</u>	
CAS #	4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the presence and absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 8, 15, 30 ug/ml With metabolic activation Study A: 4, 5, 6 ug/ml With metabolic activation Study B: 6, 7, 8 ug/ml
Metabolic Activation	Yes (Aroclor-1254 treated rat liver homogenate). Each batch of +S9 was characterized by its ability to metabolize 2-aminoanthracene and benzo(a)pyrene to forms mutagenic to <i>s. typhimurium</i> .
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	Benzo(a)pyrene: 12.5 ug/ml used with metabolic activation. N-methyl-N'-nitro-N-nitrosoquandine: 0.5 ug/ml used without metabolic activation.
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO ₂ in air for 20-24 hours. For activation assays cells were treated in suspension to a reaction mixture of NADP, NADH, NADPH, S-9 and test or control material prior to seeding. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci. The cytotoxic effects of each treatment condition were expressed relative to the

	<p>solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.</p>
<u>Results</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation. 3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
Remarks	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 49%, 66% and 95% at 30, 15 and 8.0 ug/ml respectively. No Type II or Type III transformed foci were observed at any dose level tested. One spontaneous Type III focus was observed in the solvent control. The positive control induced 6 Type II and 17 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p> <p>In the presence of metabolic activation (+S9), relative to solvent control, cell survival was 65%, 79% and 95% at 6, 5 and 4 ug/ml respectively (Study A) and 0%, 0% and 55% at 8, 7, and 6 ug/ml (Study B). A repeat assay was performed in an attempt to obtain higher levels of toxicity. Type II and Type III foci were observed in the treated cultures as follows:</p> <p>Initial Assay (+S9) 4 ug/ml: 1 Type II; 3 Type III 5 ug/ml: 5 Type II; 4 Type III 6 ug/ml: 2 Type II; 1 Type III</p> <p>Repeat Assay (+S9) 6 ug/ml: 0 Type II; 2 Type III 7 ug/ml: toxic 8 ug/ml: toxic</p> <p>The transformation frequency of the 5 ug/ml dose group was statistically significantly greater than the solvent control.</p> <p>No spontaneous Type III foci were observed in the solvent control in the initial or repeat assay. The positive control induced 9 Type II and 6 Type III foci in the initial assay and 6 Type II and 7 Type III foci in the repeat assay. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p>

<u>Conclusions</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation.</p> <p>3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/24/01 (RTA-)

Robust Summary 5-GenTox-14

<u>Test Substance</u>	
CAS #	84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the presence and absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 16, 24 and 32 ug/ml With metabolic activation: 14, 16, 18 and 20 ug/ml
Metabolic Activation	Yes (Aroclor-1254 treated rat liver homogenate). Each batch of +S9 was characterized by its ability to metabolize 2-aminoanthracene and benzo(a)pyrene to forms mutagenic to <i>s. typhimurium</i> .
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	Benzo(a)pyrene: 12.5 ug/ml used with metabolic activation. N-methyl-N'-nitro-N-nitrosoquandine: 0.5 ug/ml used without metabolic activation.
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO ₂ in air for 20-24 hours. For activation assays cells were treated in suspension to a reaction mixture of NADP, NADH, NADPH, S-9 and test or control material prior to seeding. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.

	<p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.</p>
<u>Results</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation. 3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
Remarks	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 15%, 46% and 95% at 32, 24 and 16 ug/ml respectively. One spontaneous Type III focus was observed in the solvent control. The positive control induced 20 Type II and 21 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test. Type II and Type III foci were observed in the treated cultures as follows:</p> <p>Dose Level (-S9) 16 ug/ml: 0 Type II; 3 Type III 24 ug/ml: 0 Type II; 2 Type III 32 ug/ml: 0 Type II; 1 Type III</p> <p>The transformation frequencies of the test material treated groups were not statistically increased when compared to that of the solvent control.</p> <p>In the presence of metabolic activation (+S9), relative to solvent control, cell survival was 13%, 19%, 47% and 95% at 20, 18, 16 and 14 ug/ml respectively. Two Type II and one Type III spontaneous foci were observed in the solvent control. The positive control induced 5 Type II and 14 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test. Type II and Type III foci were observed in the treated cultures as follows:</p> <p>Dose Level (+S9) 14 ug/ml: 2 Type II; 1 Type III 16 ug/ml: 0 Type II; 4 Type III 18 ug/ml: 3 Type II; 6 Type III 20 ug/ml: 2 Type II; 5 Type III</p> <p>The transformation frequencies of the test material treated groups at 18 and 20 ug/ml were statistically increased when compared to that of the solvent control.</p>

<u>Conclusions</u>	<p>3T3 cell transforming activity was not observed under the conditions of this study in the absence of metabolic activation.</p> <p>3T3 cell transforming activity was observed under the conditions of this study in the presence of metabolic activation.</p>
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/25/01 (RTA-)

Robust Summary 5-GenTox-15

<u>Test Substance</u>	
CAS #	113706-15-3
Chemical Name	Phosphorodithioic acid, secondary butyl and isooctyl) mixed esters, Zn Salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	18, 27, 36 ug/ml
Metabolic Activation	No
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine: 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control</p>

	must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.
<u>Results</u>	3T3 cell transforming activity was observed under the conditions of this study in the absence of metabolic activation at extremely toxic doses. The Study Director concluded that the test material should be considered suspect for transforming activity in this assay.
<u>Remarks</u>	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 5%, 49% and 89% at 36, 27 and 18 ug/ml, respectively. No spontaneous foci were observed in the solvent control. The positive control induced 16 Type II and 27 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p> <p>Type II and Type III foci were observed in the test material treated cultures as follows:</p> <p>Dose Level (-S9) 18 ug/ml: 0 Type II; 1 Type III 27 ug/ml: 1 Type II; 2 Type III 36 ug/ml: 1 Type II; 3 Type III</p> <p>The transformation frequency of the 36 ug/ml dose group was statistically significantly greater than that of the solvent control.</p>
<u>Conclusions</u>	3T3 cell transforming activity was observed under the conditions of this study in the absence of metabolic activation at extremely toxic doses. The Study Director concluded that the test material should be considered suspect for transforming activity in this assay.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/25/01 (RTA-)

Robust Summary 5-GenTox-16

<u>Test Substance</u>	
CAS #	68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Schechtman, L., Kouri, R., Progress in Genetic Toxicology, Elsevier/North-Holland Biomedical Press, New York, pp. 307-316 (1977).
Test Type	Morphological transformation of BALB/3T3 mouse embryo cells in the absence of metabolic activation
GLP (Y/N)	Y
Year (Study Performed)	1982
Test System	BALB/3T3 mouse embryo cells
Exposure Method	Dilution
Test Substance Doses/concentration levels	30, 45, 60 ug/ml
Metabolic Activation	No
Vehicle	The test material was solubilized in acetone and diluted to the appropriate concentration in complete Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin-streptomycin. Solvent control plates were treated with acetone at the same concentration needed to expose the target cells to the highest dose of test article in complete medium.
Positive Control concentration level	N-methyl-N'-nitro-N-nitrosoguanidine: 0.5 ug/ml
Statistical Analysis	The transforming potential of each treatment condition was compared to that of the solvent control using the Poisson distribution.
Test Substance Solubility	Test substance was solubilized in acetone.
Dose rangefinding study	Yes
Remarks field for test conditions	<p>Exponentially growing 3T3 clone A31-1 cells were seeded for an evaluation of cytotoxicity at 250 cells/culture in triplicate/condition, and at 1×10^4 cells/plate in 12-15 replicates for the determination of phenotypic transformation. Plates were incubated at 36° in a humidified atmosphere of 5% CO₂ in air for 20-24 hours. Cells were exposed to three concentrations of test article as well as to solvent and positive controls for 24 hours. Cells were then washed and the treated media replaced with untreated growth medium. After 7-10 days incubation, the concurrent cytotoxicity plates were fixed with methanol, stained with 10% Giemsa, and scored for colony formation. After 4-6 weeks incubation with twice weekly medium changes, the transformation plates were fixed with methanol, stained with 10% Giemsa, and scored for morphologically transformed Type II and Type III foci.</p> <p>The cytotoxic effects of each treatment condition were expressed relative to the solvent treated control (relative cloning efficiency). The transformation frequency for each treatment group was expressed as the number of transformed foci/surviving cell. For a valid test the cloning efficiency of the solvent control must be greater than or equal to 25%. The relative survival of cells exposed to the test article must fall within the range of 30-60% for one dose level and 60-90% for another dose level. The number of Type III foci in the negative control</p>

	must not exceed one-focus/total replicate plates. The positive control must induce a significant ($p \leq 0.05$) number of Type III foci relative to the negative control.
<u>Results</u>	Substantial, though not statistically significant, 3T3 cell transforming activity was observed, under the conditions of this study, in the absence of metabolic activation at moderate to non-toxic dose levels. The Study Director concluded that this test material should be considered suspect of transformation activity in this assay.
<u>Remarks</u>	<p>In the absence of metabolic activation (-S9), relative to solvent control, cell survival was 1%, 31% and 67% at 60, 45 and 30 ug/ml respectively. One spontaneous Type III focus was observed in the solvent control. The positive control induced 21 Type II and 26 Type III foci. Based on these results the negative and positive controls fulfilled the requirements for the determination of a valid test.</p> <p>Type II and Type III foci were observed in the test material treated cultures as follows: Dose Level (-S9) 30 ug/ml: 1 Type II; 0 Type III 45 ug/ml: 1 Type II; 4 Type III 60 ug/ml: 1 Type II; 0 Type III</p>
<u>Conclusions</u>	Substantial, though not statistically significant, 3T3 cell transforming activity was observed, under the conditions of this study, in the absence of metabolic activation at moderate to non-toxic dose levels. The Study Director concluded that this test material should be considered suspect of transformation activity in this assay.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/25/01 (RTA-)

Robust Summary 5-GenTox-17

<u>Test Substance</u>	
CAS #	4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, 0.0016, 0.0012, 0.00089, 0.00067 ul/ml. With metabolic activation: Assay A: 0.021, 0.016, 0.012, 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, or 0.0016 ul/ml. With metabolic activation: Assay B: 0.022, 0.021, 0.020, 0.019, 0.018, or 0.017 ul/ml. With metabolic activation: Assay C: 0.021, 0.018, or 0.017 ul/ml. With metabolic activation: Assay D: 0.021, 0.016, 0.012, 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, or 0.0016 ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and

	water. Acetone was selected as the appropriate vehicle.
Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK⁻ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter</p>

	<p>and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 35 to 98% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 3 to 100% total growth.</p> <p>None of the nonactivated cultures exhibited mutant frequencies that were significantly greater than the mean mutant frequency of the solvent controls.</p> <p>The highest dose tested in the S-9 activated cultures exhibited a mutation frequency that was more than 2X the mean mutation frequency of the solvent controls. Based on this result three additional assays were conducted as follow-up studies with S-9 activation. In the first +S9 repeat assay total growth ranged from 3 to 71%. Some contamination was present and complete results were obtained from 11 of 18 cultures. These eleven cultures exhibited mutation frequencies that were significantly greater than the mutation frequency of the solvent control (2.2-9.4X solvent control). This assay was repeated due to the contamination and an erratic dose-response. In the second +S9 repeat assay total growth ranged from 3 to 44%. These cultures exhibited mutation frequencies that were significantly greater than the mutation frequency of the solvent control (3.1-11.3X solvent control). In the third +S9 repeat assay a second lot of test material was used. Total growth ranged from 27 to 96%. None of These cultures exhibited mutation frequencies that were significantly greater than the mutation frequency of the solvent control. However, a dose dependent increase in mutation frequency was noted (1.0-1.9X solvent control). Based on the results of this series of experiments it was concluded by the Study Director that the test material produced an equivocal response in the presences of metabolic activation.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>

<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/19/01 (RTA-0)

Robust Summary 5-GenTox-18

<u>Test Substance</u>	
CAS #	26566-95-0
Chemical Name	Phosphorodithioic acid, mixed 0-(2-ethylhexyl)- O-(2-methylpropyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, 0.0016, 0.0012, 0.00089, 0.00067 ul/ml. With metabolic activation: 0.021, 0.016, 0.012, 0.0089, 0.0067, 0.005, 0.0038, 0.0028, 0.0021, or 0.0016 ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and water. Acetone was selected as the appropriate vehicle.

Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK⁻ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter</p>

	<p>and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 11 to 98% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 59 to 101% total growth.</p> <p>None of the nonactivated cultures exhibited mutant frequencies that were significantly greater than the mean mutant frequency of the solvent controls.</p> <p>One +S9 culture (0.021 ul/ml) that was cloned exhibited a mutation frequency that was 2X the mean mutant frequency of the solvent controls. The total growth of this culture was 59%. None of the remaining +S9 cultures that were cloned exhibited mutation frequencies that were significantly greater than the mean mutation frequency of the solvent controls. The total growth of these cultures ranged from 78 to 101%.</p> <p>Based on these results it was concluded by the Study Director that the test material produced an equivocal response in the presences of metabolic activation.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>
<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation and produced an equivocal response in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/20/01 (RTA-0)

Robust Summary 5-GenTox-19

<u>Test Substance</u>	
CAS #	113706-15-3
Chemical Name	Phosphorodithioic acid, secondary butyl and isooctyl) mixed esters, Zn Salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.024, 0.018, 0.013, 0.010, 0.0075, 0.0042, 0.0032, 0.0024 or 0.0018 ul/ml. With metabolic activation: 0.061, 0.049, 0.036, 0.023 or 0.01ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and water. Acetone was selected as the appropriate vehicle.

Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 1, 0.1, 0.01 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK^{-/-} cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity</p>

	<p>plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	The test substance was not mutagenic in this assay without metabolic activation and produced a positive response in the presence of metabolic activation.
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 2 to 97% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 8 to 95% total growth.</p> <p>One of the nonactivated cultures (0.024 ul/ml) that was cloned exhibited a mutation frequency that was 2.8X the mean mutation frequency of the solvent controls. The total growth of this culture was 2%. None of the remaining cultures exhibited mutation frequencies that were significantly greater than the mean mutant frequency of the solvent controls. The total growth of these cultures ranged from 19 to 97%. The Study Director did not consider this single culture significant since mutation frequencies observed at such highly toxic levels may be due to epigenetic events.</p> <p>The +S9 cultures treated at 0.061, 0.049, 0.036, 0.023 ul/ml exhibited mutation frequencies that ranged from 13.2 to 2.0 times the mean mutant frequency of the solvent controls. The total growth of these cultures ranged from 8 to 84%. None of the remaining +S9 cultures that were cloned exhibited mutation frequencies that were significantly greater than the mean mutation frequency of the solvent controls. The total growth of these cultures ranged from 86 to 95%.</p> <p>Based on these results it was concluded by the Study Director that the test material produced a positive response in the presences of metabolic activation.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>
<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation and produced a positive response in the presence of metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/23/01 (RTA-0)

Robust Summary 5-GenTox-20

<u>Test Substance</u>	
CAS #	68457-79-4
Chemical Name	Phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1983
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	L5178Y cells, which were actively growing in culture, were cleansed. Three ml of THMG (Thymidine, Hypoxanthine, Methotrexate, Glycine) were added to 100 ml cell suspension containing 0.1×10^6 cells/ml. Culture was gassed with 5% CO ₂ in air and incubated at 37°C at 125 rpm for 24 hours. After 24 hours the THMG was removed and the cells were rinsed in 20 ml of Fisher's Media with 0.1% Pluronic with 10% heat inactivated serum (F10P) and reinstated in culture at 3×10^4 cells/ml in 100 ml of F10P plus 1 ml of THG stock solution. Cell population density of the prepared cultures was determined by adding a 1 ml sample of cells to 9 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts/sample with an electronic cell counter. A cell suspension containing 1×10^6 cells/ml was then prepared and 6 ml aliquots were dispensed to polypropylene centrifuge tubes.
Exposure Method	Dilution
Test Substance Doses/concentration levels	Without metabolic activation: 0.013, 0.010, 0.0075, 0.0056, 0.0042, 0.0032, 0.0024, 0.0018 or 0.0013 ul/ml. With metabolic activation: 0.024, 0.018, 0.013, 0.010, 0.0075, 0.0056, 0.0042, 0.0032, 0.0024 or 0.0018 ul/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	Acetone
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 7.5 and 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 1.0 and 0.5 ul/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Three counts/plate were made using an automatic colony counter. The median count was recorded. Plates were counted by hand if necessary. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies ($\times 10^4$) in the corresponding vehicle control plates and multiplying by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined during a solubility test that compared the solubility of the test material in acetone, DMSO, ethanol and water. Acetone was selected as the appropriate vehicle.

Toxicity Determination	<p>A preliminary toxicity test with and without S-9 activation was conducted. Cultures were initiated by seeding one tube/dose and two/solvent control with 6 ml of cell suspension from a common pool containing 1×10^6 cells/ml. The test material was solubilized and diluted for testing at 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 ul/ml with S-9. In the absence of S-9, the test material was prepared at 100, 10, 1, 0.1, 0.01 and 0.001 ul/ml. The test material was added to each culture tube. Four ml of Fishers media or S-9 activation mixture were added to each tube. The tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 24 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer.</p> <p>Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material by removing 1 ml samples from each culture, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes and counting the samples with an electronic cell counter.</p>
Mutagenicity Assay (Remarks field for test conditions)	<p>This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not performed. This deviation from the guideline was not considered sufficient to invalidate this study.</p> <p>Based on the toxicity determination the test material was prepared so that the highest concentration was 100% toxic. The test material was solubilized and serial dilutions were carried out. Dose solutions were produced and cultures were treated in triplicate. The test material was added to the cultures along with S-9, as appropriate, in amounts at which the final solvent concentration was nontoxic to the cells. Final cell suspensions were 0.6×10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation.</p> <p>All tubes were gassed with 5% CO₂ in air and incubated at 37°C at 25 rpm for 4 hours. Cultures were prepared under amber light and kept in darkness during incubation. After 4 hours the test material was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting. The cells were washed twice in 10 ml F10P, resuspended in 20 ml of F10P, gassed with 5% CO₂ in air and replaced on the mixer at 37°C for two days. Cell population adjustments were made at 24 and 48 hours to yield a cell population of 0.3×10^6 cells/ml. The cells were then plated in a restrictive media containing 3 ug/ml trifluorothymidine (TFT) which allows TK⁻ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability.</p> <p>Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. Three counts/plate were made on an automatic colony counter and the median count was recorded. The frequency of mutation by dose was determined by comparing the average number of colonies in the mutagenicity</p>

	<p>plates to the average number of colonies in the corresponding viability plates.</p> <p>For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 0.2 to 1.0/10⁴ cells; negative control plating efficiency should be at or above 50%.</p> <p>The following criteria were used as guidelines in judging the significance of test material activity: Positive – if there is a positive dose response and one or more of the three highest dose levels exhibit a mutation frequency two-fold greater than background. Equivocal – no dose response but any one or more dose levels exhibit a 2x increase in mutation frequency over background. Negative - no dose response and none of the dose levels exhibit a 2x increase in mutation frequency over background.</p>
<u>Results</u>	<p>The test substance was not mutagenic in this assay in the absence of metabolic activation. The results in the presence of metabolic activation did not permit an accurate assessment of the compounds mutagenic potential.</p>
Remarks	<p>Nonactivated cultures were cloned over a range of concentrations that produced 2 to 92% total growth. The S-9 cultures were cloned over a range of test article concentrations that produced 6 to 125% total growth.</p> <p>Two of the nonactivated cultures (0.013 and 0.010 ul/ml) that were cloned exhibited mutation frequencies that were 10.8 and 2.5X the mean mutation frequency of the solvent controls. The total growth of these cultures was 2 and 4%. None of the remaining cultures exhibited mutation frequencies that were significantly greater than the mean mutant frequency of the solvent controls. The total growth of these cultures ranged from 20 to 92%. The Study Director did not consider these two cultures significant since mutation frequencies observed at such highly toxic levels may be due to epigenetic events.</p> <p>The +S9 culture treated at 0.024 ul/ml exhibited a mutation frequency that was 7.2 times the mean mutant frequency of the solvent controls. The total growth of this culture was 6%. None of the remaining +S9 cultures that were cloned exhibited mutation frequencies that were significantly greater than the mean mutation frequency of the solvent controls. The total growth of these cultures ranged from 77 to 125%. The total growth of the +S9 treated cultures did not cover the critical range of survival (10-40%) desired for this assay. The test material induced a precipitous toxic response. The cultures treated at the two highest concentrations of test material had 6% and 77% total growth. The Study Director concluded that a repeat assay was not appropriate since the difference in dose concentration between the two highest concentration cultures was only 0.006 ul/ml. The Study Director further concluded that the assay results, in the presence of metabolic activation, did not permit an accurate assessment of the test materials mutagenic potential.</p> <p>Positive and vehicle control group responses were appropriate and met the criteria outlined above.</p>

<u>Conclusions</u>	The test substance was not mutagenic in this assay without metabolic activation. Reliable results were not available in the presence of metabolic activation.
<u>Data Quality</u>	Reliable with restriction (Klimisch Code) Restriction due to the lack of a reliable result in the assay conducted with metabolic activation.
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 4/23/01 (RTA-0)

2.4 Toxicity to Reproduction

Robust Summary 5-ReproTox-1

<u>Test Substance</u>	
CAS #	CAS# 4259-15-8
Chemical Name	2-ethyl hexyl derivative
Remarks	Test material purity not provided.
<u>Method</u>	
Method/Guideline followed	OECD 421
Test Type	Oral reproductive/developmental toxicity screening study
GLP (Y/N)	Y
Year (Study Performed)	1994
Species	Rat
Strain	Sprague-Dawley CD, 71 days of age at initiation of treatment
Route of administration	Orally by gastric intubation
Duration of test	Fo males- 28 days total (14 days premating; 14 day mating period) Fo females- at least 43 days total (14 days premating; mating; 25 days of gestation and 4 days of lactation. F ₁ pups- gestation plus 4 days of lactation.
Doses/concentration levels	0, 30, 100 and 200 mg/kg/day
Vehicle control	Mazola® Corn Oil
Dose volume	5 mL/kg
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity and weekly dose concentration confirmation.
Control and treatment groups	12 Fo rats/sex/group in the control, low, mid and high dose groups.
Post exposure observation period	None
Mating ratio	One male to one female
Duration of mating period	Up to 10 days with initial male; if positive evidence of mating not present (sperm or copulatory plug) then female paired with a second proven breeder male from the same dose group for up to five additional days.
Statistical methods	Pup ratios, pup survival indices, mean number stillborn and dead pups and parental fertility indices were evaluated using the Chi-square test with Yates correction factor. Fo body weights and gains, gestation and lactation body weights and gains, parenteral food consumption, mean litter weights, length of gestation, live litter size and organ weights were evaluated using ANOVA (two-tailed) with Dunnett's test. Histopathological findings were evaluated using the Kolmogorov-Smirnov (one-tailed) test. Data obtained from nongravid animals were excluded from statistical analysis following the mating period. The litter was the experimental unit.
Dose rangefinding study	None

Remarks field for test conditions	<p>All Fo animals were dosed for a minimum of 14 days prior to mating and through the day of necropsy. All animals were examined twice daily for appearance and behavior. Body weights were recorded weekly for both sexes prior to mating; maternal body weights were recorded on gestation days 0, 7, 14 and 20 as well as on lactation days 1 and 4. Food consumption was recorded for corresponding intervals prior to mating, during gestation and lactation. Animals were paired 1:1 for mating. Positive evidence of mating was confirmed by the presence of sperm or a vaginal copulatory plug (day 0 of gestation). If evidence of mating was not present after 10 days, the female was placed with a second male from the same group for 5 days. The second male was a proven breeder based on a prior successful mating. All of the surviving Fo females were allowed to deliver and rear their pups to lactation day 4. The offspring were potentially exposed <i>in utero</i> and through nursing during lactation days 1-4 until euthanization on post-natal day 4. The surviving Fo dams were necropsied on lactation day 4, following at least 43 days of dosing. The surviving Fo males were necropsied after the breeding period, following 28 days of dosing. The Fo females with total litter loss were necropsied within 24 hours. Fo females that failed to deliver were necropsied on post-mating day 25 (with evidence of mating) or 25 days following the termination of the mating period (with no evidence of mating). Organ weights were collected for all Fo animals and microscopic examinations were conducted for all control and high dose animals and for all parental animals not surviving to their scheduled necropsy. Tissues examined microscopically included the epididymides, cervix, coagulation gland, ovaries, pituitary, prostate, seminal vesicles, testes, uterus, vagina vas deferens and gross lesions.</p> <p>Offspring dying between days 0-4 of lactation were necropsied. Carcasses were processed for possible skeletal evaluation. Litters were examined daily. Pups were individually weighed on lactation days 1-4 and were sexed on lactation days 0-4. All surviving pups were euthanized and necropsied on post-natal day 4 with emphasis placed on developmental morphology.</p>
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Results

Fo (Parental Generation)

Two males and three females in the high dose group (200 mg/kg/day) died prior to scheduled necropsy (test days 12, 19, 8, 27 and 39).

These deaths were considered treatment related. Two females in the mid dose group (100 mg/kg/day) and one female in the high dose group were euthanized on lactation days 1 or 2 due to total litter loss. Five of these animals exhibited gastric irritation upon necropsy. All other animals survived to their scheduled sacrifice.

Clinical signs noted in the found dead or sacrificed animals included staining, matting of fur, respiratory distress, hunched appearance and mucoid diarrhea. Clinical findings noted for the surviving mid and high dose males and females included post dosing salivation, brown staining, respiratory distress and diarrhea. No treatment related clinical findings were observed in the low dose (30 mg/kg/day) animals.

Fertility indices (%) for the high dose males and females were slightly lower than control as follows:

	<u>Control</u>	<u>30 mg/kg</u>	<u>100 mg/kg</u>	<u>300 mg/kg</u>
Males	91.7	83.3	83.3	81.8
Females	100	83.3	91.7	81.8

These values were within the range of the test facility historical control data (64-100%). In addition, differences from control were not statistically significant and represent only 1 or 2 fewer successful matings out of 11 or 12 males or females used for mating in the control and high dose groups. A microscopic examination of the reproductive organs of these animals did not reveal any treatment-related effects. The Study Director concluded that the low and mid dose groups were unaffected and that these data did not clearly reflect a treatment related effect in the high dose group. Other reproductive parameters (mating indices, days between pairing and coitus, gestation length and parturition) were unremarkable in all treated groups.

The pre-mating (weeks 1-4) mean body weight gain of the high dose males was statistically significantly reduced compared to control. The mean body weights of the low and mid dose males and all treated female groups were unremarkable during the pre-mating period. Gestation and lactation body weights were unremarkable in all treated groups. Food consumption data were unremarkable in all treated groups (males and females) during the pre-mating, gestation and lactation periods. With the exception of the gastric irritation noted above in several unscheduled deaths, the macroscopic data were unremarkable. Absolute and relative (to body weight) organ weight data as well as the microscopic examination data of the Fo males and females were unremarkable. There were no treatment-related findings evident in any of these data.

F₁ Litter Data

Pup body weights, live litter size and sex ratios were unremarkable. No treatment related effects were evident. An increased number of

	<p>dead pups was noted in the mid dose group on day 0 of lactation. Pup viability indices in the mid dose (lactation days 1 and 4) and high dose (lactation day 4) groups were reduced. This was attributed to total litter loss by three females. Increased pup deaths were observed in the mid and high dose groups during the post-natal period. An increased incidences of pups without milk in the stomach was noted in the mid dose group. No treatment related effects were evident in the necropsy data of these found dead pups or in the necropsy data from scheduled pup necropsies.</p> <p>Chemical analysis of dosing suspensions confirmed that they were homogeneous and of appropriate concentration throughout the study.</p>
Remarks	
<u>Conclusions</u>	Parental (Fo) toxicity was exhibited at dose levels of 100 (mortality, clinical findings) and 200 mg/kg/day (mortality, clinical signs, reduced body weight gain, gastric irritation). A slightly reduced fertility index was also observed at 200 mg/kg/day. No Fo toxicity was observed at 30 mg/kg. Neonatal (F ₁) toxicity (mortality) was observed at 100 and 200 mg/kg/day. No F ₁ toxicity was observed at 30 mg/kg. Based on these findings the Study Director concluded that the NOAEL for both parental and neonatal toxicity was 30 mg/kg/day.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 3/14/00 (RTA-030)